Quorum Sensing and Plant-Induced Gene Expression in the Novel Group of Plant Beneficial and Environmental *Burkholderia*

Thesis

Quorum Sensing and Plant-Induced Gene Expression in the Novel Group of Plant Beneficial and Environmental *Burkholderia*

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"It is good to have an end to journey toward; but it is the journey that matters, in the end."

— Ernest Hemingway

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Abstract

The genus *Burkholderia* is composed of functionally diverse species and it can be divided into several groups. One of these, designated as the plant-beneficial-environmental (PBE) *Burkholderia* group, is formed by non-pathogenic species, which in most cases have been found to be associated with plants. It was previously established that members of the PBE group share an *N*-acyl-homoserine lactone (AHL) quorum-sensing (QS) system designated BraI/R that produces and responds to 3-oxo-C_{14}-HSL (OC14-HSL). In the present study, we further studied the BraI/R system in several members of the PBE group determining the AHL production profile as well its regulons. Major results include that different levels of AHLs are produced by different species and that the regulon is species specific. The biosynthesis of exopolysaccharide was the only common phenotype found to be regulated by BraI/R in several species of the PBE cluster. In addition, BraI/R was shown not to be important for plant nodulation by *B. phymatum* spp. nor for endophytic colonization and growth promotion of maize by *B. phytofirmans* PsJN. Moreover, the genome of the rice endophyte *B. kururiensis* M130 was sequenced and analysed in order to detect potential loci involved in its endophytic lifestyle and plant growth promotion. Two experimental approaches (plate screening of a transposon-promoter probe library and RNAseq) were then performed in order to identify loci that were regulated in response to plant macerate. The results indicated that *B. kururiensis* undergoes major regulatory changes affecting the expression of 27.7% of its protein coding genes. Interestingly, a great number of differentially expressed genes encode membrane transporters and secretion systems, which indicates that the exchange of molecules is an important aspect *in planta*. In addition, genes related to mobility, chemotaxis and adhesion were also over-represented suggesting recognition and an intimate interaction between bacteria and plant. This work highlights the close signalling taking place between plants and bacteria and helps us to understand the adaptation of an endophyte *in planta*.

Publications

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Table of Contents

Index of Figures

Index of Tables

Abreviations List

ACP: acyl carrier protein

AHL: N-acyl-homoserine lactones

BLAST: basic local alignment search tool

Tc: tetracycline

Km: kanamycin

Nf: nitrofurantoin

CFU: colony forming units

Rif: rifampicin

YEM: yeast extract mannitol

SAM: *S*-adenosylmethionine

NSA: nutrient-sucrose agar

LC-ESI-MS/MS: liquid chromatography-electrospray ionization-multi-stage/mass spectrometry

HPLC: high performance liquid chromatography

X-Gal: 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

Amp: ampicilin

DSF: diffusible signal factor

LB: Luria Bertani

KB: King's B

ncRNA: noncoding ribonucleic acid

asRNA: antisense ribonucleic acid

PGPB: plant growth promoting bacteria

T3SS: type III secretion system

T6SS: type VI secretion system

EPS: exopolysaccharide

GST: glutathione-S-transferase

COG: clusters of orthologous groups

PHA: polyhydroxyalkanoates

PHB: poly-B-hydroxybutyrate

ISR: induced systemic resistance

2,4,5-T: 2,4,5-trichlorophenoxyacetic acid

PCB: polychlorobiphenyl

TCE: trichloroethylene

OD: optical density

ORF: open reading frame

TLC: thin layer chromatography

X-GlcU: 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid

MLST: multilocus sequence typing

PBE: plant beneficial and environmental

BCC: *Burkholderia cepacia* complex

CF: cystic fibrosis

NS: not statistically different

ND: not determined

LNR: low number of reads

PAMP: pathogen-associated molecular patterns

RND: resistance nodulation and cell division

ACC: 1-aminocyclopropane-1-carboxylate

IAA: indole-3-acetic acid

BNF: biological nitrogen fixation

LPS: lipopolysaccharide

QS: quorum sensing

1. Introduction

1.1 The Burkholderia Genus

1.1.1 General features

The *Burkholderia* genus consists of Gram-negative, rod-shaped, β-proteobacteria species that are motile by means of one or more polar flagella. The majority of the approximately 75 recognized species comprising this genus is aerobic and use oxygen as the terminal electron acceptor during respiratory metabolism; some species are anaerobic and utilize nitrate as electron acceptor for respiration. *Burkholderia* are catalase positive and are able to degrade a wide variety of carbon compounds possessing great nutritional versatility (Palleroni, 2005).

The species belonging to this genus are recognized by their ability to colonize remarkably diverse ecological niches. They have been isolated from soil, water, industrial and hospital environments; moreover they are able to colonize plants, animals, humans and fungi (Coenye and Vandamme, 2003). Recently, *Burkholderia* spp. were found at high densities in the midguts of stinkbugs (*Riptortus clavatus*) forming host-specific symbiotic associations (Kikuchi et al., 2011). Some of these endosymbiotic *Burkholderia* spp. confer insecticide resistance to their insect hosts by degrading fenitrothion, an organophosphate insecticide (Kikuchi et al., 2012). This great niche diversity has provided many *Burkholderia* spp. with interesting biotechnological properties such as ability to degrade recalcitrant xenobiotics and/or promote plant growth by enhancement of disease resistance, contribution to better water management, improvement of nitrogen fixation, etc (Coenye and Vandamme, 2003; Nowak and Shulaev, 2003; Compant et al., 2005a; Sessitsch et al., 2005; Ait Barka et al., 2006; Barrett and Parker, 2006; Janssen, 2006; Balandreau and Mavingui, 2007). However, the genus *Burkholderia* also contains opportunistic or obligate pathogens of plants, animals and humans, which makes their possible use in agriculture and biotechnology more difficult (Coenye and Vandamme, 2003; Compant et al., 2008).

1.1.2 Taxonomy of the genus *Burkholderia*

Walter H. Burkholder was an American plant pathologist who described one of the first *Burkholderia* species, *Phytomonas caryophylli* (Burkholder, 1942) as a carnation pathogen. Eight years later, he also described a phytopathogenic bacterium

able to cause sour skin in onion and named it *Pseudomonas cepacia* derived from the Latin name for onion, *cepia* (Burkholder, 1950). This species would then be designated as *Burkholderia cepacia* and become the first type species of the *Burkholderia* genus which was proposed by Yabuuchi *et al.* (1992).

Since the creation of the *Burkholderia* genus many new species have been described. Their identification and taxonomy is complex and requires a polyphasic approach which includes different diagnostic tests (Payne et al., 2005). Moreover, the *Burkholderia* genus varies functionally and, several studies and phylogenetic trees have evidenced the presence of distinct divisions among members of this genus. (Payne et al., 2005; Payne et al., 2006; Tayeb et al., 2008; Onofre-Lemus et al., 2009). These studies based on *recA*, *16SrDNA, gyrB, rpoB* and *acdS* genes showed the presence of two major groups (Figure 1.1) with bootstraps values higher than 90. This division separates the *Burkholderia cepacia* complex (BCC), the "pseudomallei group", the phytopathogenic species and the endosymbionts of phytopathogenic fungi from the non-pathogenic *Burkholderia* species that establish beneficial or neutral interaction in association with plants or the environment (Suárez-Moreno et al., 2012; Estrada-de los Santos et al., 2013).

This division seems to reflect their natural environments since all the species already reported as pathogenic or potentially pathogenic clustered separately from the ones that are harmless or beneficial. Studies of multilocus sequence typing (MLST) and whole genome comparisons have shown that species of *Burkholderia* that are part of the plant-associated-nitrogen fixing clade (*e.g. B. xenovorans*) locate distantly from the BCC species and "pseudomallei group" (Spilker et al., 2009; Ussery et al., 2009; Vanlaere et al., 2009; Estrada-de los Santos et al., 2013). All these results suggest that members of the *Burkholderia* genus fall into two different groups, one that comprises all the potentially pathogenic species and the other one that contains the species associated with plants or found in the environment. The latter group is currently often referred to as the Plant Beneficial and Environmental group (PBE group) (Suárez-Moreno et al., 2012).

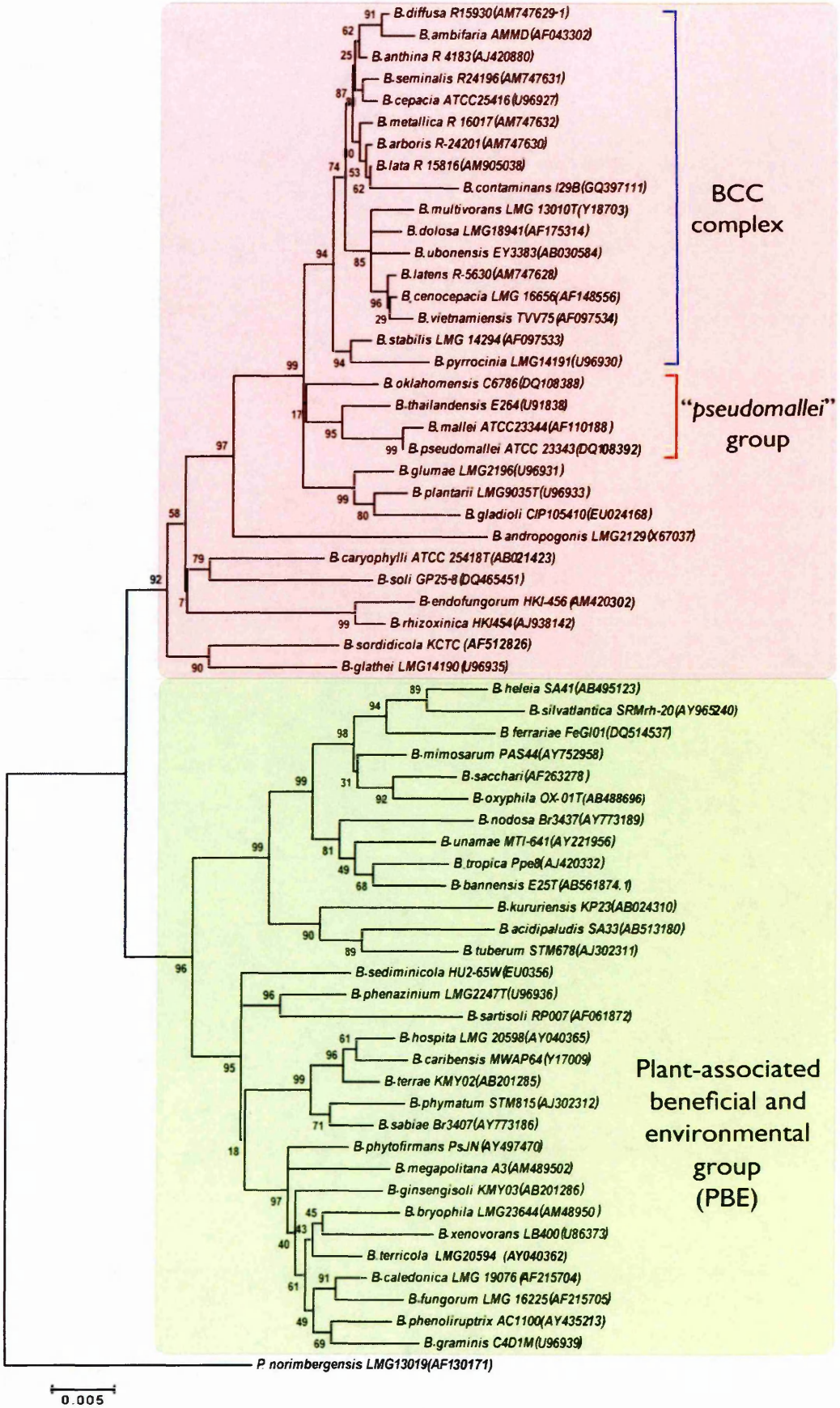
B.diffusa R15930(AM747629-1)
B.ambifaria AMMD(AF043302)
B.anthina R 4183(AJ420880)
B.seminalis R24196(AM747631)
B.cepacia ATCC25416(U96927)
B.metallica R 16017(AM747632)
B.arboris R-24201(AM747630)
B.lata R 15816(AM905038)
B.contaminans I29B(GQ397111)
B.multivorans LMG 13010T(Y18703)
B.dolosa LMG18941(AF175314)
B.ubonensis EY3383(AB030584)
B.latens R-5630(AM747628)
B.cenocepacia LMG 16656(AF148556)
B.vietnamiensis TVV75(AF097534)
B.stabilis LMG 14294(AF097533)
B.pyrrocinia LMG14191(U96930)
BCC complex
B.oklahomensis C6786(DQ108388)
B.thailandensis E264(U91838)
B.mallei ATCC23344(AF110188)
B.pseudomallei ATCC 23343(DQ108392)
"pseudomallei" group
B.glumae LMG2196(U96931)
B.plantarii LMG9035T(U96933)
B.gladioli CIP105410(EU024168)
B.andropogonis LMG2129(X67037)
B.caryophylli ATCC 25418T(AB021423)
B.soli GP25-8(DQ465451)
B.endofungorum HKI-456(AM420302)
B.rhizoxinica HKI454(AJ938142)
B.sordidicola KCTC(AF512826)
B.glathei LMG14190(U96935)
B.heleia SA41(AB495123)
B.silvatlantica SRMrh-20(AY965240)
B.ferrariae FeGl01(DQ514537)
B.mimosarum PAS44(AY752958)
B.sacchari(AF263278)
B.oxyphila OX-01T(AB488696)
B.nodosa Br3437(AY773189)
B.unamae MTI-641(AY221956)
B.tropica Ppe8(AJ420332)
B.bannensis E25T(AB561874.1)
B.kururiensis KP23(AB024310)
B.acidipaludis SA33(AB513180)
B.tuberum STM678(AJ302311)
B.sediminicola HU2-65W(EU0356)
B.phenazinium LMG2247T(U96936)
B.sartisoli RP007(AF061872)
B.hospita LMG 20598(AY040365)
B.caribensis MWAP64(Y17009)
B.terrae KMY02(AB201285)
B.phymatum STM815(AJ302312)
B.sabiae Br3407(AY773186)
B.phytofirmans PsJN(AY497470)
B.megapolitana A3(AM489502)
B.ginsengisoli KMY03(AB201286)
B.bryophila LMG23644(AM48950)
B.xenovorans LB400(U86373)
B.terricola LMG20594 (AY040362)
B.caledonica LMG 19076(AF215704)
B.fungorum LMG 16225(AF215705)
B.phenoliruptrix AC1100(AY435213)
B.graminis C4D1M(U96939)
Plant-associated beneficial and environmental group (PBE)
P.norimbergensis LMG13019(AF130171)
0.005

Figure 1.1. Phylogenetic tree based on 16S rRNA sequences of the recognized species of the *Burkholderia* genus (Suárez-Moreno et al., 2012).

1.1.3 The "potentially pathogenic *Burkholderia* group"

In 1997, Vandamme and coworkers performed a polyphasic taxonomic study and demonstrated that bacteria identified as "*B. cepacia*" consisted of at least five closely related, but distinct genomic species designated genomovars (Vandamme et al., 1997). All these different genomovars were part of the newly created clade called the BCC and further taxonomy then assigned species names to these genomovars (Coenye et al., 2001a). The BCC now comprises 18 species (see Table 1.1) that share high levels of 16S rRNA (>97.5%) and *recA* (94-95%) gene sequence similarities and moderate (30–60%) DNA–DNA hybridization values (Coenye et al., 2001a; Vanlaere et al., 2008b; Vanlaere et al., 2009).

In the beginning of the 1980s *B. cepacia* was identified in cultures of respiratory tract specimens from cystic fibrosis (CF) patients (Isles et al., 1984; Tablan et al., 1985), the most common fatal genetic disease of Caucasians. These reports described the virulent nature of the infection by this species, leading to a rapidly progressive necrotizing pneumonia and sepsis in about 20% of the patients, a clinical outcome that was designated as cepacia syndrome. Another major problem associated with BCC infection in CF patients is their inherent resistance to antimicrobial treatment which is possibly exacerbated due to the presence of these bacteria in biofilms (McClean and Callaghan, 2009). Although all the 18 BCC species have been isolated from the lungs of CF patients in heterogeneous frequencies depending on the geographic localization, the most commonly isolates are *B. cenocepacia*, and *B. multivorans* (Mahenthiralingam et al., 2008; McClean and Callaghan, 2009; Vial et al., 2011).

Table 1.1. Bacterial species comprised in the "potentially pathogenic *Burkholderia* group" and their characteristics.

Burkholderia spp.*	Host/Habitat	Interaction
Cepacia complex		
B. cepacia	Human, animals, rhizosphere, soil, water	Pathogen/Beneficial/ Environmental
B. cenocepacia	Human, animals, rhizosphere, plants, soil, water	Pathogen/Environmental
B. multivorans	Human, rhizosphere, soil, water	Pathogen/Environmental
B. stabilis	Human, rhizosphere	Pathogen/Environmental
B. vietnamiensis	Human, animals, rhizosphere, plants, soil, water	Pathogen/Beneficial/ Environmental
B. dolosa	Human, rhizosphere, plants	Pathogen/Environmental
B. ambifaria	Human, rhizosphere, soil	Pathogen/ Beneficial/ Environmental
B. anthina	Human, rhizosphere, soil	Pathogen/Environmental
B. pyrrocinia	Human, rhizosphere, plants, soil, water	Pathogen/Environmental
B. ubonensis	Human, soil	Pathogen/Environmental
B. latens	Human	Pathogen
B. diffusa	Human, soil, water	Pathogen/Environmental
B. arboris	Human, rhizosphere, soil, water	Pathogen/Environmental
B. seminalis	Human, rhizosphere	Pathogen/Environmental
B. metallica	Human	Pathogen
B. contaminans	Human, animals, soil, water	Pathogen/ Beneficial/ Environmental
B. pseudomultivorans	Human, rhizosphere	Pathogen/Environmental
B. lata	Human, soil, water	Pathogen/Environmental
***pseudomallei* group**		
B. mallei	Human, animals	Pathogen
B. pseudomallei	Human, soil, water	Pathogen/Environmental
B. thailandensis	Human, soil	Pathogen/Environmental
B. oklahomensis	Human, soil	Pathogen/Environmental
Phytopathogens		
B. andropogonis	Sorghum, clover, carnation, orchids	Pathogen
B. caryophylli	Carnation, Russel prairie gentian	Pathogen
B. gladioli	Gladiolus, rice, tulips, mushrooms, animals, human	Pathogen
B. glumae	Rice, human	Pathogen
B. plantarii	Rice	Pathogen

Burkholderia spp.*	Host/Habitat	Interaction
Endosymbionts of phytopathogenic fungi		
B. rhizoxinica	*Rhizopus microsporus*	Endosymbiont
B. endofungorum	*Rhizopus microsporus*	Endosymbiont
B. sordidicola	*Phanerochate sordida*	Endosymbiont
***B. glathei* group**		
B. glathei	Soil	Environmental
B. zhejiangensis	Wastewater-treatment system, human	Environmental/Pathogen
B. grimmiae	*Grimmia montana*	Environmental
B. humi	Soil	Environmental
B. choica	Soil	Environmental
B. telluris	Soil	Environmental
B. udeis	Soil	Environmental
B. terrestris	Soil	Environmental
B. symbiotica	Root nodules of *Mimosa* spp.	Beneficial
B. soli	Soil	Environmental

*References on the bacterial species can be found in (Coenye and Mahenthiralingam, 2014) and therein.

It is important to highlight that some BCC species are also readily isolated from natural environments, mainly from the rhizosphere of several plants (*e.g.* maize, rice, pea) (Mahenthiralingam et al., 2008; Vial et al., 2011). For instance, *B. vietnamiensis* TVV75 was isolated from rice and is able to support rice plant growth, increasing the grain yield. Similarly, *B. ambifaria* MC17 is able to control maize diseases caused by *Fusarium moniliforme* (Bevivino et al., 2000; Trân Van et al., 2000). Many BCC species are able to produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase that lowers the plant ethylene levels contributing to plant growth. In addition, many species also produce a wide variety of antifungals and other compounds that can prevent the colonization of the plant by microbial pathogens (Vial et al., 2007; Onofre-Lemus et al., 2009; Vial et al., 2011). The natural environment is therefore one of the main sources for infection of CF and immunocompromised patients by *Burkholderia* spp. as clonally identical BCC strains can be found in both niches. For this reason, their use as bio-inoculant agents in agriculture is no longer allowed (Mahenthiralingam et al., 2008; Vial et al., 2011).

The "pseudomallei group" is composed of *B. pseudomallei*, *B. mallei*, *B. thailandensis*, and *B. oklahomensis*. *B. pseudomallei* is the etiologic agent of the human disease melioidosis, a febrile illness that can range from an acute pneumonia or septicemia to chronic abscesses, after the exposure to soil or water. This disease is most commonly reported in Southeast Asia and Northern Australia and because of the lethal and contagious nature of the disease, *B. pseudomallei* is listed by the Centers for Disease Control (CDC) as a potential bioterrorism agent (Inglis and Sagripanti, 2006; Lazar Adler et al., 2009). *B. thailandensis* was isolated from the environment as a *B. pseudomallei*-like species, but there are only few documented cases of human infectious diseases associated with *B. thailandensis* and they usually involve immunocompromized patients (Brett et al., 1998; Glass et al., 2006b). The reason for the disease attenuation of *B. thailandensis* in comparison with *B. pseudomallei* has been in part associated with the presence of a functional arabinose biosynthesis operon in *B. thailandensis*, which is almost completely deleted in *B. pseudomallei*. Studies showed that the introduction of the complete operon into *B. pseudomallei* results in the downregulation of several type III secretion genes and a reduced virulence of the strain in Syrian Hamsters (Moore et al., 2004).

B. mallei is an obligate mammalian pathogen and is the etiologic agent of glanders in horses, mules, donkeys and humans, in which the infections involve sepsis, pneumonia and abscess formation with a high mortality rate. This pathogen is endemic in many parts of the world, including Asia, Africa, the Middle East and South America and no successful vaccines have thus far been developed. Like *B. pseudomallei*, *B. mallei* is also listed by the CDC as a potential agent for biological warfare and it has been already used as such during the American Civil War, World War I and II and the Russian invasion of Afghanistan (Whitlock et al., 2007). Another species of the "pseudomallei group" is *B. oklahomensis* which has been isolated from purulent wounds of patients accidentally exposed to ground soils in Oklahoma and is the least virulent species of the group (Glass et al., 2006a; Wand et al., 2011).

Several *Burkholderia* spp. are plant pathogens (Table 1.1). *B. gladioli* for example was initially identified as a pathogen of *Gladiolus*, causing rot of gladiolus corms, but it was already associated with diseases in other plants, such as onions, freesia, tulip and rice and the disease symptoms may vary from the spotting of foliar

parts to scabbing and rotting of storage tissues (McCulloch, 1921; Matsuyama, 1998; Ura et al., 2006). Although *B. gladioli* is better known for its phytopathogenic interactions, some strains have also demonstrated the ability to infect animals, including humans, causing food poisoning and even severe chronic pulmonary infections in CF patients (Wilsher et al., 1997; Jones et al., 2001; Jiao et al., 2003; Foley et al., 2004). *B. gladioli* is also a pathogen in mushrooms and can cause soft rotting symptoms on several commercially important mushrooms, such as *Lentinula edodes*, *Pleurotus ostreatus*, *Flammulina velupies* and in different cultivated *Agaricus* species (Gill and Tsuneda, 1997; Roy Chowdhury and Heinemann, 2006). *B. glumae* is one of the most important rice (*Oryza sativa*) pathogens causing panicle blight through the production of toxoflavin and lipase, which are the major virulence factors of this species discovered thus far (Ham et al., 2011). *B. glumae* causes wilt in other crops, including pepper, eggplant, tomato and sesame (Jeong et al., 2003). Analogously, *B. plantarii* causes seedling blight of rice by the production of a toxin called tropolone, which is responsible for root growth inhibition and wilting of the seeds (Azegami et al., 1987; Urakami et al., 1994; Coutinho et al., 2014).

Other *Burkholderia* species are reported to be endosymbionts of phytopathogenic fungi (Partida-Martinez and Hertweck, 2005). Endosymbionts of *Rhizopus microsporus B. endofungorum* or *B. rhizoxinica* synthesize mycotoxins that enable the fungus to cause rice seedling blight. These toxins can weaken or even kill the plant, providing nutrients for both the fungus and its endosymbiont (Partida-Martinez and Hertweck, 2005; Partida-Martinez et al., 2007). *B. sordidicola* lives within the fungal cells of *Phanerochaete sordida*, a white-rot phytopathogen that inhabits fallen branches of hardwood trees (Lim et al., 2003). Until now there are no evidences that *B. sordidicola* contributes to the pathogenicity of its host (Compant et al., 2008).

The *B. glathei* group is the only one composed of mainly environmental species that were never associated with pathogenic interactions, with the only exception being *B. zhejiangensis*. The ecological niche and role of most of the species of this group remain however unknown (Coenye and Vandamme, 2003). *B. glathei* was the first species of the group to be isolated and it was recovered from fossil lateritic soils in Germany (Zolg and Ottow, 1975). As *B. glathei*, the majority of the species belonging to this group was isolated from different types of soils, however there have not been

many strains identified belonging to these species, therefore it is possible that, in the near future, with the isolation of more strains, their role will be elucidated and they are likely to be revealed as phytopathogenic agents (Compant et al., 2008).

1.1.4 The "Plant Beneficial and Environmental *Burkholderia* Group"

Although the *Burkholderia* genus is notorious for its pathogenicity to both humans and plants as highlighted above, the majority of the plant-associated species are nonpathogenic as their interactions with their hosts or with the environment are beneficial or neutral, but not detrimental (see Table 1.2). Members of the phylogenetically distinct PBE group are mostly associated with plants and also commonly found in sediments and bulk soil. Some of them are able to colonize the exterior and interior of plant organs in an intimate association that can lead to the promotion of plant growth, enhancement of the host's resistance to biotic and abiotic stress and/or conversion of nitrogen to ammonia via biological nitrogen fixation (BNF). In addition, most of them are catabolically versatile, being able to degrade recalcitrant compounds. Recent studies showed that members of PBE group lack important functions required for mammalian and plant pathogenesis which suggests that their risk of causing opportunistic infections is extremely low (Angus et al., 2014). These features highlight the biotechnological potential of this group of *Burkholderia*, as they can be used as bioinoculants in agriculture and bioremediation (Compant et al., 2008; Suárez-Moreno et al., 2012).

Table 1.2. Bacterial species comprised in the "plant beneficial and environmental *Burkholderia* group" and their characteristics

Species*	Isolated from	Host/Habitat	Relevant characteristics
B. acidipaludis	Endophyte, rhizosphere	*Eleocharis dulcis*	*nif*+, aluminum tolerant
B. bannensis	Rhizosphere	*Panicum repens*	*nif*+
B. bryophila	Associated with mosses	*A. Palustre, S. palustre, S. rubellum*	PGPB, antifungal activity
B. caledonica	Endophyte, Rhizosphere, soil	*Vitis vinifera,* Rubiaceae	*acdS*
B. caribensis	Nodules, vertisol	*Mimosa diplotricha*	*nif*+, *nod*+, *acdS*, high EPS production
B. ferrariae	Soil	Iron ore	*nif*+, phosphate solubilizer
B. fungorum	Fungal endosymbiont, wastewater	*Phanerochaete chrysosporium*	*acdS*, aromatic compound degradation
B. graminis	Rhizosphere	*Zea mays, L. deliciosus, P. pinea, S. lycopersicum*	*acdS*, ISR against salt and dought
B. ginsengioli	Rhizosphere	*Panax ginseng*	β-galactosidase activity
B. heleia	Rhizosphere	*E. dulcis*	*nif*+, grow in acidic environments
B. hospita	Soil	Agricultural soil	Acceptor of plasmids pJP4, pEMT1
B. kururiensis	Endophyte, Rizosphere, TCE soil	*Oryza sativa, Manihot sculenta, Nicotiana tabacum*	*nif*+, *acdS*, PGPB, aromatic compound degradation
B. megapolitana	Associated with mosses	*A. palustre*	PGPB, antifungal activity
B. mimosarum	Nodules	*M. pigra, M. pudica, M. scrabella*	*nif*+, *nod*+
B. nodosa	Nodules, soil	*M. bimucronata, M. Scrabella*	*nif*+, *nod*+, antifungal and antibacterial activity
B. oxyphila	Acidic soil	Acidic forest soil	Catabolizes (+)-catechin into taxifolin
B. phenazinium	Soil, associated with mosses	*S. rubellum*	Produces ionidin, acidophilic
B. phenoliruptrix	Chemostat with 2,4,5-T, nodules	*Mimosa flocculosa*	Degrades phenol compounds, *nif*+, *nod*+, *acdS*
B. phymatum	Nodules	*Mimosa* spp., *P. vulgaris*	*nif*+, *nod*+, *acdS*
B. phytofirmans	Endophyte, Rhizosphere, soil	*Allium cepa, Solanum* spp., *O. Sativa*	*acdS*, PGPR, antifungal activity, ISR against cold, degrades thiocyanate
B. sabiae	Nodules	*M. caesalpiniifolia*	*nif*+, *nod*+, PHA production
B. sacchari	Soil	*Saccharum officinarum*	PHA production

Species*	Isolated from	Host/Habitat	Relevant characteristics
B. sartisoli	Rhizosphere, PAH soil	*Z. mays*	Aromatic compound degradation
B. sediminicola	Water sediments		PHA production
B. silvatlantica	Rhizosphere, endophyte	*Z. mays, S. officinarum, A. Comosus*	*nif*+, *acdS*, PGPB
B. terrae	Soil, rhizosphere, mycosphere	*Broad-leaved soil forest, L. esculentum, Laccaria proxima*	*nif*+
B. terricola	Soil, rhizosphere	*Beta vulgaris*	*acdS*, acceptor of plasmids pJP4 and pEMT1
B. tropica	Rhizosphere, endophyte	*S. officinarum, L. esculentum, Z. mays, A. Comosus*	*nif*+, *acdS*, production of PHAs and EPS, PGPB
B. tuberum	Nodules	*Cyclopia spp., M. atropurpureum*	*nif*+, *nod*+, *acdS*
B. unamae	Endophyte, rhizosphere	*Z. mays, S. officinarum, Coffea Arabica*	*nif*+, *acdS*, phenol and benzene degradation, PGPB
B. xenovorans	Rhizosphere, PCB soil	*S. lycopersicum, C. Arabica*	*nif*+, *acdS*, PCB degradation

*References on the bacterial species can be found in (Suárez-Moreno et al., 2012) and therein; *nif*+, verified presence of *nifH* gene; PGPB, characteristics of plant-growth promoting bacteria; *acdS*, verified presence of ACC deaminase gene; *nod*+, verified plant nodulation; EPS, exopolysaccharide; ISR, able to induce systemic resistance in the host; TCE, trichloroethylene; 2,4,5-T , 2,4,5-trichlorophenoxyacetic acid; PHA, polyhydroxyalkanoates; PAH, polyaromatic hydrocarbons; PCB, polychlorobiphenyl (modified from Suárez-Moreno *et al.*, 2012).

1.1.4.1 General characteristics of the PBE group

1.1.4.1.1 Microbiological features

Most species of the *Burkholderia* genus have an optimal growth temperature of 30°C, however some species like *B. kururiensis* may also grow at 37°C (Zhang et al., 2000). The optimal pH is between 3 and 7, although tolerance to more acidic conditions has been reported in *B. oxyphila* and *B. heleia* (Aizawa et al., 2010a; Otsuka et al., 2010). Some species, like *B. tropica* and *B. sacchari* are able to accumulate poly-B-hydroxybutyrate (PHB) as reserve source of carbon. Most PBE species are not able to produce pigments; the exception is *B. phenazinium*, which produces iodinin in the presence of L-threonine (Byng and Turner, 1976; Messenger and Turner, 1983; Bramer et al., 2001; Reis et al., 2004). Most PBE species are motile by means of one or several polar flagella, except *B. kururiensis*, *B. bryophila*, *B. megapolitana* and *B. heleia*

(Zhang et al., 2000; Vandamme et al., 2007; Aizawa et al., 2010a). Their typical cellular fatty acids contain 14, 16, 17 and 18 carbon atoms ($C_{16:0}$, $C_{17:0}$ cyclo, and $C_{18:0}$ ω7c) and some species may contain two different ornithine lipids (Palleroni, 2005).

Most of the members of this group have a strictly aerobic respiratory metabolism with oxygen as terminal electron acceptor and, with the exception of *B. sartisoli*, are also able to reduce nitrate to nitrite, however they do not denitrify (Vanlaere et al., 2008a). All PBE *Burkholderia* species are chemorganotrophs, being able to use glucose, glycerol, inositol, galactose, sorbitol and mannitol as carbon source. They are also able to use other kinds of carbon source which varies between species and is used as discriminatory trait (Palleroni, 2005). Moreover, recent studies have shown that PBE *Burkholderia* are able to use oxalate as a carbon source (oxalotrophy), while plant pathogenic and human opportunistic pathogens do not possess the same characteristic (Kost et al., 2014).

1.1.4.1.2 Genomic features

The genomes of the species from the PBE group are very large, ranging from 6.5 Mbp to 9.95 Mbp, and are often made up of several replicons and megaplasmids. This is one of the reasons for their ecological versatility, together with lateral gene transfer events (Chain et al., 2006; Martinez-Aguilar et al., 2008). Their GC content varies from 61.2 to 64% (Suárez-Moreno et al., 2012). Several genome projects have been completed and the genomic profile is well conserved within the group. However *B. xenovorans* type strain ($LB400^T$) is distinct as it harbors a 1.47 megaplasmid which is not present in any other species; this plasmid may have been acquired by horizontal gene transfer (Martinez-Aguilar et al., 2008). A comparison between the genome sequence of *B. xenovorans* and a BCC isolate showed that only 44% of all genes were conserved among these two species (Chain et al., 2006). Moreover, a series of genomic analysis of flagella, chemotaxis, attachment and secretion systems revealed that the PBE *Burkholderia* spp. do not possess the virulence clusters that are present in the BCC group (Angus et al., 2014) highlighting the phylogenetic distance between PBE and BCC.

1.1.4.1.3 Exopolysaccharide production

Exopolysaccharides (EPS) are sugar polymers excreted from the cell and their function depends on the ecological niche of the bacteria. Most of the functions attributed to the EPS are of a protective nature, as it forms a hydrated, anionic and highly polymerized matrix that surrounds the cells. EPS is able to protect bacteria against different kinds of stress such as toxic molecules and desiccation and it can also act as one of the first contacts between bacteria and plant cell surface (D'Haeze et al., 2004; Kumar et al., 2007). EPS is also one of the most abundant components of the biofilm, a multicellular complex enclosed in a matrix that is formed through the interaction of bacteria with surfaces. Besides EPS, biofilms are composed also of glycoproteins, glycolipids and extracellular DNA (Rudrappa et al., 2008; Karatan and Watnick, 2009).

The most studied EPS in *Burkholderia* is cepacian, one of the heptasaccharide polymers produced by strains of the BCC group (Cerantola et al., 1996; Richau et al., 2000; Herasimenka et al., 2007; Cescutti et al., 2011). In the PBE group the structures of EPS for *B. caribensis*, *B. kururiensis* and *B. tropica* have been determined (Vanhaverbeke et al., 2003; Mattos et al., 2005; Silipo et al., 2008; Pol-Fachin et al., 2010). Although these EPS have different repeating units and monosaccharide proportions, they present some structural similarities, such as elevated levels of O-acetylation and the presence of carboxylated groups (Vanhaverbeke et al., 2003; Serrato et al., 2006; Silipo et al., 2008; Hallack et al., 2009). Further studies evidenced similarities between the EPS produced by *B. phytofirmans*, *B. graminis*, *B. phymatum* and *B. xenovorans* and the well-studied cepacian. The genomes of these four *Burkholderia* species contain two gene clusters similar to the ones involved in the cepacian synthesis, *bce-I* and *bce-II*. However, the *bce-II* of the PBE species does not carry the *bceM* and *bceU* genes, but instead have an additional gene *bceV*. The two *bce* clusters are located adjacent to each other in PBE group unlike the BCC species where they are separated by 155-314 kb interval depending on the species (Figure 1.2) (Moreira et al., 2003; Ferreira et al., 2010).

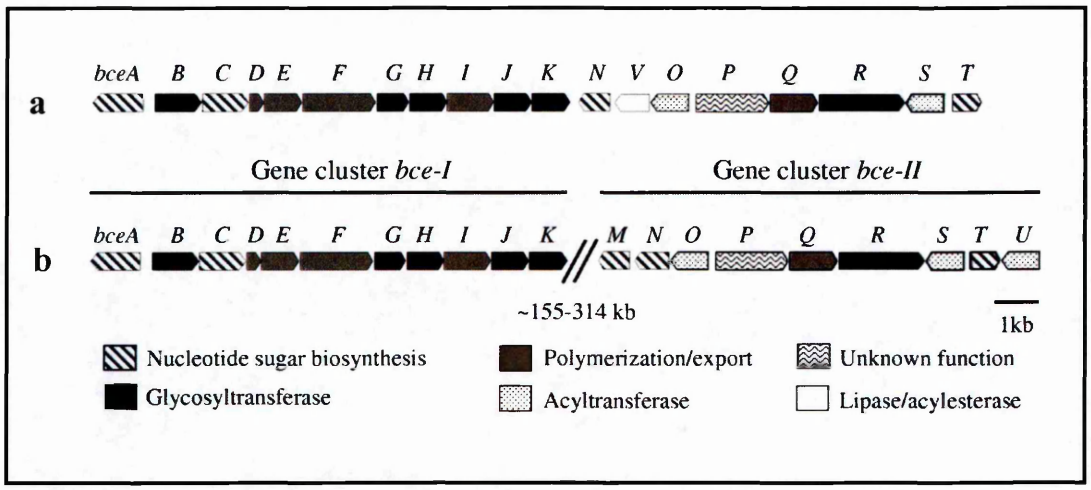

Figure 1.2. Genetic organization of the *bce* gene cluster encoding cepacian. a, species from the plant beneficial and environmental *Burkholderia* group (*B. phytofirmans*, *B. graminis*, *B. phymatum*, *B. xenovorans*); b, species from the "potentially pathogenic *Burkholderia* group" (BCC strains, *B. pseudomallei*, *B. thailandensis*, *B. oklahomensis*) (Ferreira et al., 2010).

Ferreira and coworkers (2010) have also suggested that EPS of *B. xenovorans* is important for its tolerance to desiccation and iron. In *B. caribensis*, on the other hand, EPS has been suggested to be involved in the aggregation of soils (Vanhaverbeke et al., 2003). Although it was never reported for any member of the PBE group, it is believed that EPS could be important for plant-bacteria interaction as suggested by studies with other plant beneficial bacteria. In rhizobia for example EPS plays a role in the nitrogen-fixing symbiosis and in nodule formation (Skorupska et al., 2006), while in the nitrogen-fixing endophyte *Gluconacetobacter dizotrophics* it is essential for plant colonization (Meneses et al., 2011).

1.1.4.1.4 Niches and environmental distribution

PBE *Burkholderia* can be considered ubiquitous, as they are able to survive in many different environments (Tamames et al., 2010). It is possible to find isolates of the same species from different niches in different continents. For example, *B. caribensis*

strains primarily isolated from vertisol soil in Martinique were later found in nodules of *Mimosa* spp. in China (Achouak et al., 1999; Liu et al., 2011). Species of PBE may be found free-living in the soil, associated with insects or fungi or forming interactions with plants. These interactions can be epiphytic, endophytic or endosymbiotic (Suárez-Moreno et al., 2012). Most of the *Burkholderia* from the PBE group have been isolated from the rhizosphere, which is the biologically active zone of the soil surrounding the plant roots (Table 1.2). The rhizosphere has a higher density and diversity of microorganisms due to the presence of plant-derived compounds (carbohydrates, amino acids, organic acids) released by root cells which serve as both chemotactic and nutritional substrates (Badri et al., 2009; Compant et al., 2010). The catabolic versatility of the PBE *Burkholderia* for these plant-derived compounds might account for their common occurrence in the rhizosphere (Chain et al., 2006).

Strains of *B. tropica*, *B. silvatlantica*, *B xenovorans* and *B. unamae* were repeatedly isolated from the rhizosphere of plants, suggesting that this may be their main niche (Estrada-de Los Santos et al., 2001; Caballero-Mellado et al., 2004; Perin et al., 2006b; Perin et al., 2006a; Caballero-Mellado et al., 2007). Some PBE have thus far been most commonly isolated in specific areas/countries, for example *B. unamae* has been isolated from the rhizosphere of different plants, such as coffee, maize, tomato and sugarcane in Mexico; interestingly it has not yet been isolated from many of these plants in Brazil (Caballero-Mellado et al., 2004; Perin et al., 2006b; Caballero-Mellado et al., 2007).

Rhizospheric PBE strains possess unique properties depending on the diversity of their natural habitat. For example, strains of *B. xenovorans* have been isolated from the rhizosphere of tomato and coffee, and also from a polychlorobiphenyl (PCB) polluted soil showing versatile biodegrading ability (Bopp, 1986; Goris et al., 2004; Caballero-Mellado et al., 2007). Similarly, *B. caledonica* has been isolated from the rhizosphere of *Vitis vinifera*, and from sandy soils (Coenye et al., 2001b), but also from the rhizosphere and as an endophyte of Rubiaceae, both in Europe and in Africa (Verstraete et al., 2014). On the latter study it was revealed that, although endophytic and rhizospheric *B. caledonia* are similar, geographical location separates them into genetically different groups (Verstraete et al., 2014). Furthermore, *B. phymatum* can be found in the rhizosphere of tomato plants and can also nodulate legumes being able to

fixate nitrogen also *ex planta* (Elliott et al., 2007b; Wong-Villarreal and Caballero-Mellado, 2010).

Certain PBE species can move from the rhizosphere to the internal tissues of the plants living as endophytes. Endophytes are defined as microorganisms that live inside plants without harming them (Hardoim et al., 2008). Some endophytes are able to spread systemically inside the plant, colonizing stems, leaves and, in some cases, even flowers and fruits (Hardoim et al., 2008; Compant et al., 2010). Endophytes can also have plant growth-promoting effects and/or protect plants from invading pathogens (Compant et al., 2010). *B. tropica*, *B. kururiensis*, *B. unamae*, *B. silvatlantica*, *B. phytofirmans*, and *B. acidipaludis* have all been isolated from surface-sterilized plant tissues and therefore are considered putative endophytes (Frommel et al., 1991; Baldani et al., 1997b; Caballero-Mellado et al., 2004; Reis et al., 2004; Aizawa et al., 2010b).

B. kururiensis M130 is one of the best characterized endophytic strains from the PBE group and it can increase rice growth and yield (Baldani et al., 1997a; Baldani et al., 2000). This strain is a diazotroph isolated from Brazil and it was shown to increase the nitrogen availability to the plant, which may be one of the reasons for its plant-growth promotion ability (Baldani et al., 2000). Interestingly, strain *B. kururiensis* KP23^T which was isolated from an aquifer polluted with trichloroethylene (TCE) in Japan for its ability to degrade this toxic compound (Zhang et al., 2000), can also endophytically colonize rice plants (Mattos et al., 2008).

PBE *Burkholderia* are also found in the symbiotic nodules of legumes. To capture nitrogen, legumes are able to form beneficial symbioses with different species of bacteria. These bacteria are collectively called rhizobia and they can convert atmospheric nitrogen gas (N_2) into ammonia. This symbiosis results in the development of root nodules, where these bacteria fix atmospheric nitrogen, making it available for the plants in exchange for carbon compounds (Oldroyd et al., 2011). Until recently, it was believed that only members of the α-proteobacteria were able to nodulate legumes, as bacteria from other classes, like the β-proteobacteria, were considered to be exclusively free-living nitrogen fixers and/or loosely associated with plants. However, nodulation of *Mimosa* spp. by *Burkholderia* spp. and *Cupriavidus* spp. revealed the existence of β-rhizobia (Chen et al., 2003; Gyaneshwar et al., 2011). Seven *Burkholderia* from the PBE group have now been reported as legume symbionts, these

are: *B. phymatum*, *B. tuberum*, *B. nodosa*, *B. mimosarum*, *B. caribensis*, *B. sabiae* and *B. phenoliruptrix* and they were isolated from *Machaerium lunatum*, *Aspalathus carnosa*, *Mimosa* spp., *Rhynchosia ferufolia* and *Cyclopia* spp. (Moulin et al., 2001; Chen et al., 2003; Chen et al., 2005; Chen et al., 2006; Chen et al., 2007; Elliott et al., 2007a; Elliott et al., 2007b; Chen et al., 2008; Garau et al., 2009; de Oliveira Cunha et al., 2012). Although legume-nodulating *Burkholderia* were identified much later, Bontemps and coworkers (2010) (Bontemps et al., 2010) demonstrated that the symbiosis-related genes from these *Burkholderia* were not recently acquired from other legume-nodulating bacteria and that they are ancient symbionts of legumes, possibly existing since the time when nodulation was first evolving in legumes.

Besides living in bulk soil, in the rhizosphere, endophytically or forming nodules with plants, one species of the PBE *Burkholderia* group has been isolated in association with a mycorrhizal fungus. *B. terrae* was recovered from the mycosphere of *Laccaria proxima* and further studies showed that this *Burkholderia* is able to degrade almost all of the fungal compounds released by the mycorrhiza (Warmink and van Elsas, 2008, 2009; Warmink et al., 2011).

1.1.4.2 The biotechnological potential of the PBE group

One of the most interesting characteristics of the PBE group of *Burkholderia* is their great biotechnological potential. These *Burkholderia* present characteristics of plant growth promoting bacteria (PGPB), as they are able to colonize plants and improve their growth. They are also good bio-degraders, being able to reduce the concentration and toxicity of recalcitrant compounds in the environment (Suárez-Moreno et al., 2012).

PGPB can improve plant growth by one or more direct and indirect mechanisms, such as (1) increasing mineral solubilization and nitrogen fixation, making nutrients available for the plant; (2) producing phytohormones such as indole-3-acetic acid (IAA) and/or enzymes like 1-aminocyclopropane-1-carboxylate (ACC) deaminase; (3) inducing systemic resistance in plants against biotic and abiotic stress; (4) suppressing pathogens by the production of siderophores, antibiotics and/or nutrient competition (Glick, 1995; Gupta et al., 2000).

1.1.4.2.1 Phosphate solubilization and Nitrogen fixation

Phosphorus and nitrogen are the mineral nutrients required in the greatest amounts and their availability is an imperative limiting factor for plant growth, as they are major components of many important structural, metabolic and genetic molecules. Phosphorus is present in soil in the form of insoluble phosphate salts, which cannot be absorbed by plants. Similarly, molecular nitrogen (N_2) accounts for 80% of the atmospheric composition and plants are not able to utilize it (Sashidhar and Podile, 2010; Kraiser et al., 2011). Many soilborne microorganisms can solubilize insoluble phosphorus complex and make it available for the plant (Lugtenberg and Kamilova, 2009; Sashidhar and Podile, 2010). This ability has been observed in *B. tropica* and *B. ferrariae*, however more studies need to be performed in order to evaluate their potential use in bioinoculation (Valverde et al., 2006; Caballero-Mellado et al., 2007).

The ability to fix nitrogen is a main feature of the PBE group of *Burholderia*. Studies have reported that *B. unamae*, *B. xenovorans*, *B. kururiensis*, *B. tropica*, *B. heleia*, *B. silvatlantica*, *B. gisengisoli* and *B. terrae* are diazotrophs associated to plants, *B. ferrariae* can also fix nitrogen, but was only isolated as free-living. *B. phymatum*, *B. tuberum*, *B. mimosarum*, *B. nodosa*, *B. sabiae*, *B. caribensis* and *B. phenoliruptrix* are able to fix nitrogen in symbiosis with legumes, as was mentioned above (Estrada-de Los Santos et al., 2001; Moulin et al., 2001; Chen et al., 2003; Caballero-Mellado et al., 2004; Reis et al., 2004; Chen et al., 2005; Perin et al., 2006b; Caballero-Mellado et al., 2007; de Oliveira Cunha et al., 2012; Suárez-Moreno et al., 2012). PCR assays of the pivotal *nifH* gene showed that the type strains of *B. bannensis* and *B. acidipaludis* possess this gene, however acetylene reduction assays and growth in N-limiting media were negative, thus additional studies need to be performed to determine their diazotrophy (Aizawa et al., 2010b; Aizawa et al., 2011). Although BNF is prevalent in species of the PBE group, it is also present in the BCC member *B. vietnamiensis* (Gillis et al., 1995; Menard et al., 2007) and in the newly identified member of the "potentially pathogenic group", *B. symbiotica* which nodulates *Mimosa* spp. (Sheu et al., 2012). Phylogenetic studies with the 16SrRNA and *nifH* genes suggested that it is highly possible that the common ancestor of all the *Burkholderia* species was a diazotroph and that this feature was lost in some of the species during evolution (Bontemps et al., 2010). *In planta* studies with some of the diazotrophic *Burkholderia* showed their great

ability to improve the nitrogen uptake by plants. One example is the endophyte *B. kururiensis* M130, which increased the total nitrogen content of rice by 30%, improving the grain yield (Baldani et al., 1997a; Baldani et al., 2000). Moreover, *B. tropica* was shown to increase the sugarcane yield and its use has been recommended in Brazil as part of a bacterial consortium (Ribeiro et al., 2010).

1.1.4.2.2 ACC deaminase and IAA production: modulation of plant hormones

Bacteria are able to improve plant growth by the production of the enzyme ACC deaminase that lowers the plant's ethylene levels. Ethylene is produced by plants during stressful conditions and is detrimental to them. The ACC molecule is a precursor of the ethylene synthesis pathway in plants, and the ACC deaminase is able to degrade it to ammonia and α-ketobutyrate and is produced by the *acdS* gene. This enzyme therefore makes the plant more resistant to growth inhibition by ethylene-inducing stresses (Glick and Stearns, 2011). The production of ACC deaminase and its role in plant growth promotion has been reported for endophytic *B. phytofirmans* and rhizospheric *B. unamae*, in which *acdS* mutants were not able to improve canola and tomato growth, respectively (Onofre-Lemus et al., 2009; Sun et al., 2009). ACC deaminase is a widely distributed feature of the *Burkholderia* genus, being present in at least 20 species, 14 of them belonging to the PBE group. These results evidence the great importance of this group of *Burkholderia* to plant growth under natural conditions (Blaha et al., 2006; Onofre-Lemus et al., 2009).

Another way in which bacteria influence plant growth is by the production of phytohormones such as auxins (IAA). When in contact with the surface of plant roots or seeds, some PGPB synthesize IAA in response to the amino acid tryptophan, which is present in plant exudates. IAA can stimulate plant cell proliferation and elongation even in the presence of abiotic stresses, such as high levels of salt (Glick and Stearns, 2011). Production of IAA has been detected in *B. kururiensis*, *B. phytofirmans* and *B. unamae*, but the biosynthesis pathway and its importance for plant growth promotion remain unknown (Caballero-Mellado et al., 2007; Sun et al., 2009).

1.1.4.2.3 Induced systemic resistance (ISR) against biotic and abiotic stresses

The ability to trigger ISR in plants has been observed in both rhizobacteria and endophytes and many bacterial traits, such as flagellation, LPSs, siderophores and volatile organic compounds, have been suggested as responsible for ISR. ISR induced by PGPB can alter host physiology and its metabolic responses, as well as fortify plant cell walls. ISR therefore enhances the synthesis of plant defense molecules when in contact with pathogens and/or abiotic stress factors (Compant et al., 2005a).

B. phytofirmans PsJN can trigger ISR in potato, tomato and grapevine, making these plants more resistant to their pathogens (Nowak et al., 1995; Ait Barka et al., 2000). Endophytic colonization of grapevine by *B. phytofirmans* stimulates host defense via accumulation of phenolic compounds and the strengthening of cell walls (Compant et al., 2005b). Moreover, this bacterium improves the ability of grapevine to withstand cold stress by modulating carbohydrate metabolism (Ait Barka et al., 2006; Fernandez et al., 2012). Another species from the PBE *Burkholderia* group that showed ISR features is *B. graminis*. This bacterium has been isolated from the rhizosphere of several plants (*e.g.* wheat, pasture, corn) and is able to enhance the neck diameter and the shoot height of tomato plants, in addition to induce systemic resistance to salt (Barriuso et al., 2005; Barriuso et al., 2008).

1.1.4.2.4 Pathogen suppression

Another mechanism by which PGPB can help plants from the attack of pathogens, is by directly antagonizing their growth by either competing for an ecological niche or by producing anti-microbial compounds (Compant et al., 2005a). One of the most important elements necessary for growth of all living organisms is iron and, generally, its bioavailability in soils and on plant surfaces is very low. Under iron-limiting conditions, PGPBs can produce molecules called siderophores that are able to bind Fe3+. As fungal siderophores most commonly have lower affinity towards Fe3+ when compared to bacterial siderophores, PGPB very often deprive pathogenic fungi of iron (Compant et al., 2005a; Lugtenberg and Kamilova, 2009). *B. bryophila* and *B. megapolitana* associated with the mosses *Aulacomnium palustre*, *Sphagnum rubellum* and *Sphagnum pallustre* in Germany, produce siderophores and display antifungal and

antibacterial activity. These characteristics make them interesting candidates for the use as biocontrol agents (Vandamme et al., 2007). Other potential biocontrol agents from PBE *Burkholderia* group are *B. phytofirmans* for its antifungal properties and *B. unamae*, *B. xenovorans* and *B. tropica* for their high production of siderophores (Sessitsch et al., 2005; Caballero-Mellado et al., 2007). Interestingly, studies have hypothesized a possible role for *B. tropica* in the biocontrol of nematodes in sugarcane. Statistical analysis suggested a negative correlation between the presence of the nematode *Xhipinema elongatum* and the bacteria and a positive correlation between *B. tropica* and less pathogenic nematodes (Omarjee et al., 2008).

1.1.4.2.5 Aromatic compound degradation

The fast industrialization of the world has created toxic-environmental pollution. The conventional physical or chemical techniques used to restore polluted sites are not always effective or environmentally friendly; in addition they can be expensive and unsafe. An alternative technique is phytoremediation, in which the plants and their associated microorganisms are used to degrade the toxic compounds (Zhuang et al., 2007; Weyens et al., 2009). Some organic compounds, when released in the environment, can persist for a long time. These include the polycyclic aromatic hydrocarbons (PAHs) which result from the exploration and use of fossil fuel and polychlorinated biphenyls (PCBs) used in industrial processes, as well as several chlorinated aromatics used in replacement of PCB, such as trichloroethylene (TCE) (Zhuang et al., 2007). Some PBE *Burkholderia* species have great potential to be used in phytoremediation; strains of *B. xenovorans*, *B. kururiensis*, *B. unamae*, *B. sartisoli* and *B. phenoliruptrix* have already been isolated from polluted soils or plant rhizosphere and have the capacity to tolerate and metabolize toxic compounds (Bopp, 1986; Zhang et al., 2000; Caballero-Mellado et al., 2004; Coenye et al., 2004; Caballero-Mellado et al., 2007; Vanlaere et al., 2008a). As previously mentioned, *B. xenovorans* LB400^T and *B. kururiensis* KP23 were isolated from a PCB-containing landfill and a TCE polluted site, respectively (Bopp, 1986; Zhang et al., 2000; Goris et al., 2004). *B. unamae* strains isolated from tomato rhizosphere have also the ability to degrade phenol and benzene (Caballero-Mellado et al., 2007). *B. sartisoli* RP007^T was isolated from a PAH contaminated soil in New Zealand, and is able to degrade low

molecular mass PAHs such as naphthalene, phenanthrene and anthracene (Laurie and Lloyd-Jones, 1999b, a; Tecon et al., 2006). *B. phenoliruptrix* AC1100 was recovered after consecutive platings of a mixed culture from a chemostat grown with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) as a sole carbon source (Kilbane et al., 1982). 2,4,5-T is a powerful herbicide and a constituent of the famous Agent Orange. Strain AC1100 can remove up to 97% of 2,4,5-T from polluted soils and is also able to degrade other halogen-substituted phenol compounds (Kilbane et al., 1982; Karns et al., 1983). Although these PBE *Burkholderia* spp. were shown to have a great biotechnological potential, molecular analysis of the gene clusters responsible for their catabolic versatility have not yet been carried out and are largely unknown, except for *B. xenovorans*.

1.1.4.3 Gene regulation in the PBE *Burkholderia* group

Although the interest towards PBE *Burkholderia* has grown in the past few years, the molecular mechanisms regulating the interactions between them, their host and the environment are still poorly understood. The most studied species in this aspect is *B. xenovorans* LB400, for its great capability in degrading PCB and other aromatic compounds. Several studies have been performed in order to elucidate the mechanisms of regulation of metabolic pathways involved in degradation of these compounds by *B. xenovorans* LB400 (Denef et al., 2006; Parnell et al., 2010; Zoradova-Murinova et al., 2012; Romero-Silva et al., 2013). For instance, Parnell and coworkers (2010) observed that, although the biphenyl pathway of *B. xenovorans* LB400 is induced, PCBs are not degraded during growth with alternative carbon sources like succinate, which indicates that the transport of biphenyl molecules or some type of post-transcriptional regulation could be limiting PCB degradation.

Most of the few studies of global gene regulation in the PBE *Burkholderia* involve studies of the cell-cell communication system called quorum sensing (QS).

1.1.4.3.1 Quorum sensing in the PBE *Burkholderia*

A bacterial population is able to modulate its behavior in response to chemical cues that provide information about its surrounding environment by a phenomenon of

cell-cell communication known as QS. QS allows a bacterium to respond to the concentration of a signal cue produced and released into the local environment, through the regulation of its gene expression (Fuqua et al., 1994; Platt and Fuqua, 2010). Although many different diffusible cues can be used in QS, the mechanism of this process is comparable in all systems. The concentration of the signal increases together with the population and when it reaches the minimal threshold level required for detection, the cognate receptor binds to it and triggers signal transduction cascades responsible for changes in gene expression (Ng and Bassler, 2009).

The most common QS cues identified thus far in Gram-negative proteobacteria are N-acyl-homoserine lactones (AHLs). A LuxI homologue protein synthesizes AHLs that at a critical threshold concentration, binds and forms a complex with a LuxR homologue protein. In most cases, the formation of this complex will expose the DNA-binding domain of LuxR, which will recognize a palindromic region localized in the promoter area of the target genes called a *lux*-box. The binding of LuxR-AHL to these DNA regions will promote the transcription of the gene via interaction of the complex with RNA polymerase. However, some LuxRs are able to bind DNA in the absence of cognate AHL, repressing their transcription. In such cases these proteins are considered repressors and the presence of the QS cue, at threshold concentrations, relieves gene repression (Churchill and Chen, 2011; Stevens et al., 2011).

AHL molecules are composed of a homoserine lactone ring with an acyl chain that may vary in length from 4 to 18 carbons. The acyl chain can also be modified by the presence or absence of oxo or hydroxyl substitutions at the C3 position, or can even present varied degrees of unsaturation. These modifications of the acyl chain provide not only specificity to the molecule, but also modulate its hydrophobicity, while the lactone ring provides a hydrophilic nature to the compound (Fuqua and Parsek, 2002; Churchill and Chen, 2011). Once produced, AHLs may diffuse passively across the membrane or may possibly be exported by active transport mechanisms, in the case of some long-chains AHLs (Churchill and Chen, 2011). In most cases, there is a positive feedback loop of the AHL QS system by upregulating the transcription of the *luxI* AHL synthase (Fuqua and Greenberg, 2002; Fuqua and Parsek, 2002). Importantly, some species harbor more than one QS system involved in coordinating their population-based responses which could be hierarchically organized. Besides the canonical

LuxI/LuxR QS systems some bacteria possess AHL QS LuxR-type proteins that do not have a cognate LuxI. This feature is very common among proteobacteria and the terms LuxR orphans and LuxR solos have been used to describe this type of LuxR receptors (Fuqua, 2006; Case et al., 2008; Subramoni and Venturi, 2009).

QS has been shown to play a determinant role among the global regulatory mechanisms in members of the BCC (Eberl, 2006). The BCC possesses a conserved AHL QS system called CepI/R, which produces and responds to *N*-octanoyl homoserine lactone (C8-HSL) and regulates virulence as well as several other important phenotypes such as biofilm formation and siderophore production (Huber et al., 2001; Venturi et al., 2004; Eberl, 2006). Moreover, certain species within the potentially pathogenic *Burkholderia* group possess more than one AHL QS system as is the case for *B. cenocepacia*, *B. vietnamiensis*, *B. mallei*, *B. pseudomallei* and *B. thailandensis* (Ulrich et al., 2004; Malott et al., 2005; Duerkop et al., 2007; Malott and Sokol, 2007; Kiratisin and Sanmee, 2008; Duerkop et al., 2009).

The production of AHLs in the PBE *Burkholderia* group was first reported in *B. phytofirmans*, *B. megapolitana* and *B. bryophila* (Sessitsch et al., 2005; Vandamme et al., 2007). An AHL screening of 27 strains from 21 species of this group not only revealed that AHLs were produced by all the species but also showed similarity in their AHL production profiles, being composed mostly by 3-oxo-HSL derivatives (Suárez-Moreno et al., 2008). Genetic studies with *B. kururiensis* M130, *B. xenovorans* LB400 and *B. unamae* MTI-641 then showed the presence of a highly conserved AHL QS *luxIR* family pair (Figure 1.3A), named BraI/R, showing more than 75% similarity among the three species. BraI/R responds preferentially to 3-oxo-C14-HSL and orthologs of this system are present in all the PBE *Burkholderia* species tested thus far (Suárez-Moreno et al., 2008; Suárez-Moreno et al., 2010).

The BraR protein of *B. kururiensis* is composed of 235 amino acids and presents approximately 40% identity to LasR and PpuR of *P. aeruginosa* and *P. putida*, respectively. The $BraI_{KUR}$ is composed by 196 amino acids and displays 50% identity to LasI and PpuI. The BraI/R QS system is also composed of an additional regulator consisted of 105 amino acids and presenting 50% identity to RsaL proteins of *P. aeruginosa* and *P. putida*. $RsaL_{KUR}$ negatively regulates the expression of BraR; negative regulation of AHL QS systems by RsaL has also been reported for the LasI/R

and PpuI/R in *P. aeruginosa* and *P. putida* respectively (Bertani and Venturi, 2004; Rampioni et al., 2006; Suárez-Moreno et al., 2008; Suárez-Moreno et al., 2010). Interestingly, the BraI/R system exhibits low similarity with the QS systems from other *Burkholderia* species (<31%), but displays high similarity to the LasI/R and PpuI/R AHL QS systems from *P. aeruginosa* and *P. putida*, respectively (Figure 1.4) (Suárez-Moreno et al., 2010).

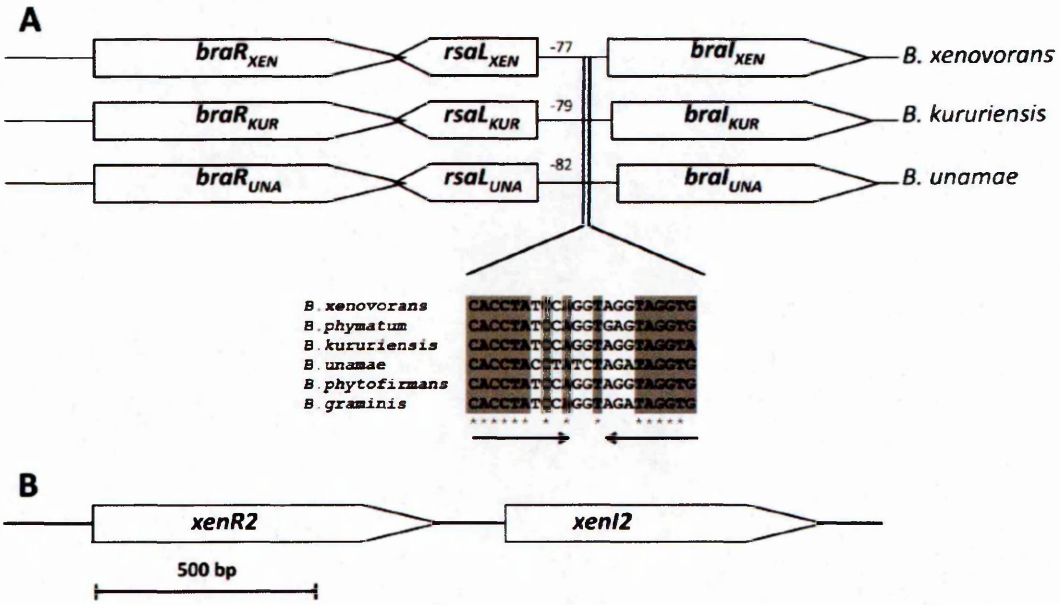

Figure 1.3. Genetic organization of the QS systems present in PBE *Burkholderia*. **(A)** Genetic maps of the *B. xenovorans* LB400^T ($braI_{XEN}$ and $braR_{XEN}$), *B. kururiensis* M130 ($braI_{KUR}$ and $braR_{KUR}$), and *B. unamae* MTl-641^T ($braI_{UNA}$ and $braR_{UNA}$) QS systems. An alignment of putative *lux* boxes is shown in several members of the PBE *Burkholderia* cluster; numbers indicate the positions upstream where the *lux* box is centered with the respect to the putative ATG start codon. **(B)** Map of the *xenI2* and *xenR2* system of *B. xenovorans* LB400^T (Suárez-Moreno et al., 2010).

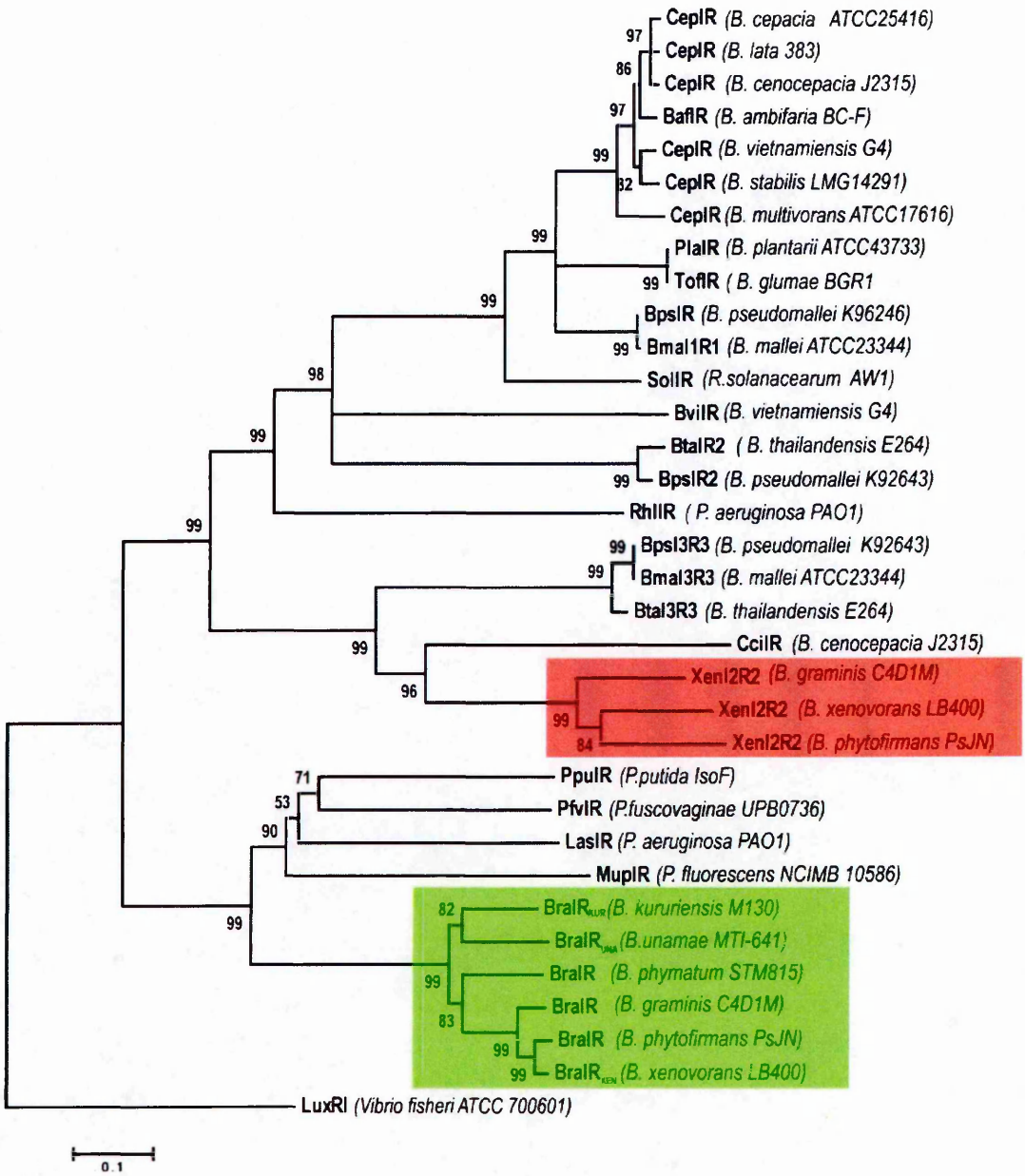

Figure 1.4. Phylogenetic analysis of the LuxIR pairs present in *Burkholderia* species. Red square shows the XenI2/R2-like systems and green square highlights the BraIR-like systems (Suárez-Moreno et al., 2012).

The presence of a common AHL QS system in all species of the PBE *Burkholderia* group tested so far suggests that this system could be part of their core genome. Studies with *B. xenovorans*, *B. kururiensis* and *B. unamae* showed that the BraI/R system is involved in the regulation of EPS production in these three species,

which might suggest the possible existence of a common regulon. However, other phenotypes such as the degradation of aromatic compounds, plant colonization and biofilm formation were found to be regulated in a species-specific manner, suggesting that the niche in which the bacteria lives might also influence in the regulon of the QS system (Suárez-Moreno et al., 2010).

Several PBE *Burkholderia* spp. possess an additional AHL QS system. *B. xenovorans* for example has an additional LuxIR pair designated XenI2/R2 (Figure 1.3B), which produces and responds to 3-hydroxy-C8-HSL.This system is more similar to the ones of other *Burkholderia* species (Figure 1.4), and is present in 6 other species of the PBE group. Interestingly, XenI2/R2 system is located in a megaplasmid in strain LB400 and is not present in all *B. xenovorans* strains, which might indicate that this QS system was acquired by lateral gene transfer events. Furthermore, studies showed that BraI/R and XenI2/R2 are not hierarchically organized (Suárez-Moreno et al., 2010).

Initial studies on QS in PBE *Burkholderia* have shown a potential role in plant-bacteria interactions. In *B. graminis* AHL QS is linked to salinity stress (Barriuso et al., 2008). In *B. phytofirmas* PsJN the XenI2/R2 QS system is important for endophytic colonization and growth promotion of *Arabidopsis thaliana* (Zúñiga et al., 2013). Similarly in *B. kururiensis*, endophytic colonization and plant growth promotion of rice requires the BraI/R QS system (Suárez-Moreno et al., 2010), however this system has only minor influence in the endophytic colonization of *A. thaliana* by *B. phytofirmans* PsJN (Zúñiga et al., 2013).

Some of the species from the PBE *Burkholderia* group were also shown to possess a diffusible signal factor (DSF)-type of QS, named BDSF (Boon et al., 2008; Deng et al., 2012). Studies with this system in *B. cenocepacia* revealed that its regulon partially overlaps with the AHL QS regulon, being also involved in regulation of biofilm formation, protease production and virulence (Deng et al., 2010; Schmid et al., 2012). Although the genes involved in BDSF production and recognition were identified in some PBE *Burkholderia* genomes (Deng et al., 2012), no functional studies have yet been performed in order to identify their role in species other than *B. cenocepacia.*

1.2 Aims and outline of this thesis

The PBE *Burkholderia* group comprises newly discovered species that present great biotechnological potential due to their ubiquity, catabolic versatility and beneficial interactions with plants. However, not much is known about the molecular mechanisms regulating these interesting characteristics. The work presented in this thesis deals mainly with gene regulation in response to QS and plant signals. One chapter of this thesis involves studies on the regulon of the conserved BraI/R QS system in different species of the PBE *Burkholderia* group and the evaluation of its role in plant-bacteria interaction. Another chapter presents the genome analysis of the rice endophyte *B. kururiensis* M130 to identify and characterize features involved in plant-bacteria interactions. The last data chapter investigated gene expression changes in response to plant macerate in the model endophyte *B. kururiensis*.

2 Regulon studies and *in planta* role of the BraI/R quorum sensing system in the plant beneficial *Burkholderia* cluster

2.1 Introduction

In the last decade, much attention has been given to β-proteobacteria in the genus *Burkholderia* mainly because of their great metabolic versatility, capacity for colonizing very distinct niches, and also due to their ability to cause serious infections in humans (Yabuuchi et al., 1992).

Currently the *Burkholderia* genus comprises over 60 validly described species; phylogenetic trees generated from diverse gene sequence analysis (*e.g.* 16S rRNA) have shown divisions within the genus, and consequently it has been separated into several clades. One clade, called the *B. cepacia* complex (BCC), is comprised of human pathogens, most abundantly isolated from the respiratory tracts of cystic fibrosis patients with chronic infections (Vandamme and Dawyndt, 2011). Another *Burkholderia* species cluster which has been recently discovered comprises non-pathogenic *Burkholderia* spp., mostly described after the year 2000, and which have been isolated from plants or from environmental samples (Suárez-Moreno et al., 2012). This latter group, also known as the plant-beneficial-environmental (PBE) *Burkholderia* group, had a strong impact on the ecological perception of *Burkholderia* spp., as it possesses several interesting features with potential biotechnological applications (Suárez-Moreno et al., 2012), including: (i) degradation of different aromatic compounds and toxic molecules; an example being *B. xenovorans* LB400 which is also able to degrade polychlorinated biphenyls (Goris et al., 2004); (ii) rhizospheric and endophytic colonization of plants combined with an ability to promote plant growth via various mechanisms, such as nitrogen fixation, 1-aminocyclopropane-1-carboxylate (ACC) deaminase and/or indole-3-acetic acid (IAA) production; an example being *B. phytofirmans* PsJN, which is able to endophytically colonize and promote plant growth of several plant varieties (Sessitsch et al., 2005); and (iii) the ability to form N_2-fixing symbioses with mosses and higher plants, providing the first confirmed examples of rhizobia belonging to the β-proteobacteria; an example being *B. phymatum*, which is able to form symbiotic nodules and fix nitrogen in association with legumes (Elliott et al., 2007b; Talbi et al., 2010).

Quorum sensing (QS) is a regulatory process that allows bacterial cells to communicate among themselves and to monitor their population density. This is possible through the production of signalling molecules which increase in accordance to

population density and, once a certain threshold is reached, bacteria sense and respond to them modulating target gene expression. The most common and studied QS system of Gram-negative bacteria thus far uses *N*-acylhomoserine lactones (AHLs) as signal molecules and is based on two genes/proteins: an AHL synthase belonging to the LuxI protein family, and a transcriptional regulator belonging to the LuxR family which detects the signal and regulates gene expression (Fuqua et al., 1994).

The first studies of QS in the PBE *Burkholderia* spp. have shown that this clade shares a highly conserved AHL QS system, called BraI/R, which is unrelated to the CepI/R present in BCC species. Besides BraI/R, another AHL QS system, called XenI2/R2, is found in only a few of the PBE *Burkholderia* spp. (*e.g. B. xenovorans*). Studies with the BraI/R QS system of *B. kururiensis* M130, *B. unamae* MTI-641 and *B. xenovorans* LB400 have revealed that it responds preferentially to 3-oxo-C_{14}-HSL (OC14-HSL) and that the XenI2/R2 of *B. xenovorans* LB400 responds to 3-hydroxy-C_8-HSL (OHC8-HSL) (Suárez-Moreno et al., 2008; Suárez-Moreno et al., 2010).

No molecular studies have yet been performed on the BraI/R gene targets. A previous study has shown that the production of exopolysaccharides (EPS) is regulated by this system in at least 3 species of the PBE clade (*B. xenovorans* LB400, *B. unamae* MTI-641 and *B. kururiensis* M130), which suggests the existence of a common BraI/R regulon. However, phenotypes such as biofilm formation, plant colonization and degradation of aromatic compounds seem to be regulated in a species-specific manner (Suárez-Moreno et al., 2010). The present work aimed to identify and compare the BraI/R QS regulons of *B. phymatum* and *B. xenovorans*, two species of the PBE cluster that occupy different environmental niches. In addition, we intended to determine the importance of this system for the plant symbiotic and endophytic life-styles of *B. phymatum* and *B. phytofirmans* PsJN, respectively.

2.2 Materials and Methods

2.2.1 Bacterial strains, plasmids and media

The *Burkholderia* spp. and *Escherichia coli* strains and plasmids used in the present study are listed in Table 2.1. The list of primers used and the construction of recombinant plasmids are given in Appendix Table 7.1. All *Burkholderia* strains were grown in King's B medium (KB) (King et al., 1954) at 30°C. *E. coli* strains were grown in Luria-Bertani (LB) medium at 37°C. *Agrobacterium tumefaciens* NTL4/pZLR4 (Shaw et al., 1997) was used for AHL detection and it was grown in LB at 30°C. The plasmid pGEM-T Easy Vector (Promega, Madison, WI, USA) was used for cloning. Antibiotics were added when required at the following final concentrations: ampicillin, 100 μg ml^{-1}; tetracycline (Tc), 15 μg ml^{-1} (*E. coli*) or 40 μg ml^{-1} (*Burkholderia* spp.); gentamicin, 30 μg ml^{-1} (*A. tumefaciens*), or; kanamycin (Km), 50 μg ml^{-1} (*E. coli*) or 100 μg ml^{-1} (*Burkholderia* spp.); and nitrofurantoin, 100 μg ml^{-1}. Conjugation experiments with *Burkholderia* spp. were performed by triparental mating using *E. coli* DH5α (pRK2013) as a helper (Figurski and Helinski, 1979) and incubated 18 h at 30°C. Transconjugants were counter-selected in KB with the appropriate antibiotics.

2.2.2 Recombinant DNA techniques

All recombinant DNA techniques were performed as described previously (Sambrook et al., 1989). Southern hybridizations were performed using Amersham Hybond-XL membranes (Amersham, Biosciences); plasmids were purified by using EuroGold columns (EuroClone, Italy); total DNA from *Burkholderia* was isolated by Sarkosyl-pronase lysis as described previously (Better et al., 1983). Generated plasmids were sequenced by Macrogen (Europe).

Table 2.1. *Burkholderia* and *E. coli* strains and plasmids used in this study

Strain or plasmid	Relevant features	Reference or source
Strains		
B. xenovorans LB400	Wild-type strain	(Goris et al., 2004)
LB400XENI2	*XenI2*::Km of *B. xenovorans* LB400	(Suárez-Moreno et al., 2010)
LB400BRAI	*braI*::Km of *B. xenovorans* LB400	(Suárez-Moreno et al., 2010)
LB400BRAR	*braR*::Km of *B. xenovorans* LB400	(Suárez-Moreno et al., 2010)
LB400BRAR ($pBBRbraR_{XEN}$)	*braR*::Km of *B. xenovorans* LB400 harboring $pBBRbraR_{XEN}$	(Suárez-Moreno et al., 2010)
B. phytofirmans PsJN	Wild-type strain	(Sessitsch et al., 2005)
PsJNBRAI	*braI*::Km of *B. phytofirmans* PsJN	This study
PsJNBRAI ($pBBRbraI_{PsJN}$)	*braI*::Km of *B. phytofirmans* PsJN harboring $pBBRbraI_{PsJN}$	This study
B. phymatum GR01	Wild-type strain	(Talbi et al., 2010)
GR01BRAI	*braI*::Km of *B. phymatum* GR01	This study
GR01BRAI ($pBBRbraI_{phym}$)	*braI*::Km of *B. phymatum* GR01 harboring $pBBRbraI_{phym}$	This study
B.phymatum STM815	Wild-type strain	(Vandamme et al., 2002)
STM815BRAI	*braI*::Km of *B. phymatum* STM815	This study
STM815BRAI ($pBBRbraI_{phym}$)	*braI*::Km of *B. phymatum* STM815 harboring $pBBRbraI_{phym}$	This study
STM815BRAI ($pMPbraI_{p}$)	*braI*::Km of *B. phymatum* STM815 harboring $pMPbraI_{p}$	This study
STM815BRAI (pMP220)	*braI*::Km of *B. phymatum* STM815 harboring pMP220	This study
STM815 ($pMPeps_{p}$)	*B. phymatum* STM815 harboring $pMPeps_{p}$	This study
STM815BRAI ($pMPeps_{p}$)	*braI*::Km of *B. phymatum* STM815 harboring $pMPeps_{p}$	This study
B. tuberum DSM17489	Wild-type strain	(Vandamme et al., 2002)
B. tuberum pME6863	*B. tuberum* DSM17489 harboring pME6863	This study
B. tropica Ppe8	Wild-type strain	(Reis et al., 2004)
B. tropica pME6863	*B. tropica* Ppe8 harboring pME6863	This study
B. terrae DSM17804	Wild-type strain	(Yang et al., 2006)
B. terrae pME6863	*B. terrae* DSM17804 harboring pME6863	This study
B. phenazinium DSM10684	Wild-type strain	(Viallard et al., 1998)
B. phenazinium pME6863	*B. phenazinium* DSM10684 harboring pME6863	This study
B. graminis DSM17151	Wild-type strain	(Viallard et al., 1998)

Strain or plasmid	Relevant features	Reference or source
B. graminis pME6863	*B. graminis* DSM17151 harboring pME6863	This study
E. coli DH5α	*F'/endA1 hsdR17 supE44 thi-1 recA1 gyrA relA1 (lacZYA-argF)U169 deoR [80dlac(lacZ)M15recA1]*	(Sambrook et al., 1989)
Plasmids		
pGEM-T Easy	Cloning vector; Amp^r	Promega
pRK2013	Tra^+, Mob^+, ColE1 replicon, Km^r	(Figurski and Helinski, 1979)
pMP220	Promoter probe vector, IncP, LacZ; Tc^r	(Spaink et al., 1987)
$pMPbraI_p$	Promoter region of *braI* from *B. phymatum* STM815 cloned into pMP220, Tc^r	This study
$pMPeps_p$	Promoter region of the EPS operon of *B. phymatum* STM815 cloned into pMP220, Tet^r	This study
pKNOCK-Km	Conjugative suicide vector; Km^r	(Alexeyev, 1999)
pKNOCK- $BRAI_{PsJN}$	Internal PCR *braI* fragment of *B. phytofirmans* PsJN cloned in pKNOCK-Km	This study
pKNOCK- $BRAI_{phym}$	Internal PCR *braI* fragment of *B. phymatum* STM815 cloned in pKNOCK-Km	This study
pME6863	*aiiA* gene under constitutive P*lac* control; Tc^r	(Reimmann et al., 2002)
pBBR1MCS-3	Broad-host-range vector; Tc^r	(Kovach et al., 1995)
$pBBRbraI_{PsJN}$	$braI_{PsJN}$ cloned into pBBR1MCS-3; Tc^r	This study
$pBBRbraI_{phym}$	$braI_{STM815}$ cloned into pBBR1MCS-3, Tc^r	This study
$pBBRbraR_{XEN}$	$braR_{XEN}$ cloned into pBBR1MCS-3, Tc^r	(Suárez-Moreno et al., 2010)

Ap^r, ampicillin resistance; Km^r, kanamycin resistance; Tc^r, tetracycline resistance.

2.2.3 Construction of gene knockout mutants and complemented strains

Genomic null mutants of the *braI* gene (encoding the AHL synthase) were created as follows: internal fragments from the *braI* genes were PCR amplified using the primers Phytof.luxI2.F/R and Phymat.luxI.F/R (Appendix Table 7.1) for *B. phytofirmans* PsJN and *B. phymatum* STM815, respectively, and cloned in pKNOCK-Km (Alexeyev, 1999). The generated plasmids, pKNOCK-$BRAI_{PsJN}$ and pKNOCK-$BRAI_{phym}$, were used as suicide delivery systems in order to create *braI* knockout mutants as previously described (Alexeyev, 1999), and the mutants thus generated were

termed PsJNBRAI and STM815BRAI, respectively. The plasmid pKNOCK-BRAI$_{phym}$ was also used to construct a *braI* knockout mutant of *B. phymatum* GR01, which was named GR01BRAI. The fidelity of all marker-exchange events was confirmed by Southern analysis or by DNA sequencing following PCR (Appendix Figure 7.1).

Complementation of the *braI* mutants was performed using the pBBRbraI$_{PsJN}$ and pBBRbraI$_{phym}$ plasmids for *B. phytofirmans* PsJN and *B. phymatum* strains, respectively. These plasmids were constructed as follows: a fragment containing the *braI* gene of *B. phytofirmans* PsJN or *B. phymatum* STM815 was amplified using the primers Phytof.braI.F/R or Phymat.braI.F/R, and cloned into pGem-T-Easy. The fragments were subsequently cloned into the high-copy plasmid pBBR1MCS-3 (Kovach et al., 1995), yielding pBBRbraI$_{PsJN}$ and pBBRbraI$_{phym}$. All plasmids were verified by sequencing (Macrogen, Europe) and then mobilized into their respective mutant strains.

2.2.4 AHL extraction and characterization

Production of AHLs was detected by TLC using AHL biosensors after extraction of AHL from cell-free spent supernatant (Shaw et al., 1997) of an overnight culture in 50 ml KB medium for most of *Burkholderia* strains and 20 ml KB medium for *B. phymatum* strains. The TLC plate was then overlaid with a thin layer of AB top agar seeded with *A. tumefaciens* NTL4/pZLR4 in the presence of 100 mg X-Gal ml^{-1} as described previously (Shaw et al., 1997).

A LC-ESI-MS/MS method was used for the analysis of AHLs extracted from the bacterial supernatants. The dried extracts were re-dissolved in 100 µL of MeOH (+0.1% v/v formic acid), and any remaining insoluble material was removed by centrifugation. Sample temperature was maintained at 4°C in the autosampler prior to analysis. The injection volume was 5.0 µL. The HPLC system used was a Shimadzu SIL-HTc autosampler with two Shimadzu LC-10ADvp pumps. The MS system used was a 4000 QTRAP hybrid triple-quadrupole linear ion trap mass spectrometer equipped with a TurboIon source. Chromatographic separation was achieved using a Phenomonex Gemini C18 reversed phase column (3.0 µm, 100 x 3.0 mm) with an appropriate guard column, maintained at 50°C, using a mobile phase flow rate set at 450 µl/min. Mobile

phases consisted of aqueous 0.1% (v/v) formic acid (A) and 0.1% (v/v) formic acid in MeOH (B). The binary gradient began initially at 10% B and ran isocratically for the first 1 min before increasing linearly to 99% B over 9 min. After a further 5 min at this composition, the gradient was returned to 10% B over the next 1 min and allowed to re-equilibrate for 4 min. The total run time was 20 min for each sample. MS detection was operated in a multiple reaction monitoring (MRM) mode, screening for all unsubstituted AHLs, 3-oxo-AHLs and 3-OH-AHLs with acyl chain lengths of 4, 6, 8, 10, 12 and 14 carbon atoms long (Ortori et al., 2011). All AHL standards used in this experiment were chemically synthesised, purified and characterised in the School of Molecular Medical Science, Centre for Biomolecular Science, University of Nottingham.

2.2.5 Determination of the AHL specificity for the BraI/R QS system of *B. phymatum* STM815

To identify the cognate AHL(s) for BraR of *B. phymatum* STM815, the promoter region of the *braI* gene was amplified with the primers PhymbraI.promF/R and cloned in the promoter-probe vector pMP220 (EcoRI-KpnI), generating pMPbraI$_p$. This plasmid was then mobilized into STM815BRAI. STM815BRAI (pMPbraI$_p$) was then inoculated into 10 ml of M9 minimal medium (Sambrook et al., 1989) supplemented with 0.2% glucose, 0.3% casamino acids, Km and Tc, grown overnight, and then diluted to OD_{600} 0.2 into 10 ml prewarmed medium (without antibiotics) containing 1 mM, 100 nM or 10 nM of the specific AHL to be evaluated. β-galactosidase activity was determined after 6 h of growth at 30°C and 180 rpm as described by Miller (Miller, 1972) with the modifications of Stachel *et al.* (Stachel et al., 1985); all experiments were performed in triplicate, and the mean value is given.

2.2.6 Total RNA isolation

RNA isolations were carried out from three independent cultures of *B. phymatum* STM815 and *B. xenovorans* LB400 and their respective QS mutants STM815BRAI and LB400BRAR. The cultures were grown in KB medium incubated at 30°C, 180 rpm until they reached an OD_{600} of 5.0 (end of logarithmic phase). RNA

isolation was carried out from 2 x 10^9 cells using the Ribopure™ bacteria RNA isolation kit (Ambion Inc., Austin, TX, USA) following the manufacturer's instructions. Isolated RNA was treated with DNase at 37°C for 1 h and purified. The purity of RNA was assessed by PCR on total RNA (250 ng) with GoTaq polymerase (Promega) using specific primers for each species. RNA quality and concentration were assessed by Nanodrop (Thermo Scientific, Wilmington, DE, USA).

2.2.7 Microarray experiment and analysis

Custom microarrays for *B. phymatum* STM815 and *B. xenovorans* LB400 wild-type and mutant strains were designed and manufactured by MYcroarray, Inc. (Ann Arbor, MI, USA) in a 40K chip based on the genome sequences of the wild-type strains (PRJNA17409 and PRJNA57823 which correspond to strain STM815 and LB400 respectively). Each microarray slide had one array composed of 40,960 potential addresses for probes, of which 37,300 or 34,604 spots contained 45-47mer probes for *B. phymatum* or *B. xenovorans* genes, respectively. The empty spaces were filled with random control probes. Each specific gene was surveyed by one unique probe sequence. There were 4 identical replicates of each *B. xenovorans* probe sequence, such that a total of 8,651 genes were surveyed by each array. For *B. phymatum* there were 5 identical replicates of each probe sequence, yielding a total of 7,460 genes being surveyed by each array. Microarray analysis was performed on three biological replicates of RNA samples collected for each strain, as described above. Labelling, hybridization and scanning were performed by MYcroarray, Inc. Briefly, slides were hybridized with cyanin-3 (Cy-3)-labelled samples and scanned with a Axon 4000B Scanner (Molecular Devices, Sunnyvale, CA, USA). Data were extracted from the scanned images using GenePix Pro Software (version 6.1.0.4). Gene functions were annotated from the National Center for Biotechnology Information (NCBI) database. Data analysis aimed at finding the differentially expressed genes (wild-type x QS mutant) was carried out using Microsoft Excel 2010. The cut-off *P*-value used was 0.05 with minimum 1.5 fold change.

2.2.8 Semi-quantitative reverse transcription polymerase chain reaction (SQ RT-PCR) and analysis

Reverse transcription was performed in a 20-μL reaction mixture containing 2.5 μg of total RNA, 200 ng of random primers/μg of RNA (Promega) and 30 U of AMV reverse transcriptase, following the manufacturer's instructions. Conditions used for RT were 65°C for 3 min, 25°C for 10 min, 42°C for 90 min and 70°C for 10 min. The primers 16s_BXE_RV/FW and Bphy_R0017-F/R were used to measure the transcription of 16S rRNA. Second-strand synthesis was performed using GoTaq Flexi polymerase (Promega) with 1 μL of undiluted (any test gene) or 1:100 diluted (16S rRNA) cDNA reaction as template. The number of PCR cycles for each gene (the primers used are listed in Appendix Table 7.2) was standardized so that the product amplification was in the linear range; 10-20 μL of the PCR product was analyzed by agarose gel electrophoresis. The intensities of the bands were measured and normalized to that of 16S rRNA using Kodak 1D software (Pizzonia, 2001) to obtain the fold difference. The validation of each gene was performed with samples from three independent isolations.

2.2.9 Mobilization of the *aiiA* lactonase gene into *Burkholderia* spp. to generate AHL-depleted strains

The pME6863 plasmid was mobilized into some PBE *Burkholderia* spp. to generate their transconjugants. The pME6863 plasmid carries the *aiiA* gene from the soil bacterium *Bacillus* sp. A24 that encodes a lactonase enzyme able to degrade AHLs (Reimmann et al., 2002).

2.2.10 EPS production and EPS promoter activity

EPS production was measured by streaking single colonies in yeast extract mannitol (YEM) or nutrient-sucrose agar (NSA) medium, as described previously (Zlosnik et al., 2008). EPS was extracted as follows: the bacterial strain was grown in 50 ml of YEM medium for 3 days at 30°C and 180 rpm; the supernatant was then separated from the bacteria through centrifugation followed by the addition of 3

volumes of cold ethanol. The mixture was left overnight at 4°C under agitation before being centrifuged at 10,000 rpm for 20 min; after centrifugation, the precipitate was left to dry and resuspended in 1 ml of sterile deionized water. The EPS was then quantified by the boiling phenol method, as described previously (Dharmapuri and Sonti, 1999).

The gene promoter of *bce-I* of *B. phymatum* STM815 was amplified with the primers PhymEPS.promF/R and cloned in the promoter-probe vector pMP220 (EcoRI-PstI), generating pMPeps$_p$. This plasmid was then mobilized into *B. phymatum* STM815 and its QS mutant, STM815BRAI. STM815 (pMPeps$_p$) and STM815BRAI (pMPeps$_p$) were inoculated into 10 ml of KB supplemented with Km and Tc, grown overnight, and then diluted to OD_{600} 0.2 into 20 ml prewarmed medium (without antibiotics). β-galactosidase activity was determined throughout different points of the growth curve, as described above. Complementation was done chemically by the addition of 10-20 μM of AHLs (mixture of 3-oxo-C_{14}-HSL, 3-oxo-C_{12}-HSL and 3-oxo-C_{10}-HSL).

2.2.11 Legume nodulation assays

Mimosa pudica cultivation and nodulation tests were carried out as described previously (Chen et al., 2005). Briefly, seeds were surface sterilized with concentrated sulphuric acid for 10 min followed by 3% sodium hypochlorite for 10 min and then washed with sterile water. Seeds were germinated on 1% water agar plates at 28°C in darkness. Nodulation experiments were carried out in tubes containing modified liquid Jensen's N-free plant nutrient medium (Somasegaran and Hoben, 1994). The seedlings were inoculated seven days after germination, with a bacterial concentration of 1 x 10^4 bacterial cells ml^{-1}. Plants were harvested 60 days after inoculation; the nodules were counted and the dry weight of the plants was measured. 10-13 plants were tested for each bacterial strain; the experiment was performed in duplicate.

Common bean (*Phaseolus vulgaris* cvs. Flamingo and Negro Jamapa) cultivation and nodulation tests were carried out as described previously (Talbi et al., 2010). Briefly, seeds were surface-sterilized with 96% ethanol for 30 s followed by immersion in 5% sodium hypochlorite for 5 min, washed with sterile water, imbibed in water for 2 h, and germinated in darkness at 30°C. Seedlings (2 per jar) were planted in autoclaved Leonard-type jars containing vermiculite and N-free nutrient solution

(Rigaud and Puppo, 1975). Plants were inoculated at sowing with approximately 10^9 cells ml^{-1} of bacteria. Plants were grown for 35 days in a greenhouse under the following conditions: 16/8 h day/night cycle and day/night temperatures of 28/20°C. 12 plants were tested for each strain and the experiment was done in duplicate (Flamingo) or triplicate (Negro Jamapa).

2.2.12 Plant endophytic colonization assays

Endophytic colonization and plant growth promotion was tested on two different maize cultivars (Kaloe and Mazurka, DOW AgroSciences Vertrebsgesellschaft m.b.H., Austria). Seeds were surface sterilized for 5 min in 70% ethanol and 5 min in 5% sodium hypochlorite solution. They were then washed five times for one minute in sterile water, and aliquots of the washing water were spread on 10% TSB agar to check for sterility. *B. phytofirmans* PsJN, PsJNBRAI and PsJNBRAI (pBBRbraI$_{PsJN}$) were grown overnight in LB medium or in LB medium supplemented with the appropriate antibiotics for the mutant strains. 5 ml of the overnight culture were used to inoculate 50 ml of fresh LB, and cultures were incubated at 28°C in a shaker until an OD_{600} of 1. Cells were washed and resuspended in fresh LB medium. Surface-sterilized seeds were soaked in bacterial suspensions for 30 min with soft shaking. Control seeds were soaked in sterile LB only. Seeds were placed on water agar (15%) and incubated in the dark at 27°C. Emerging sprouts were analyzed after 4 days of incubation. The number of germinated seeds, number of roots and length of sprout and roots were then determined.

For the analysis of endophytic colonization three emerged sprouts per treatment were surface sterilized and cut into small pieces. Plant material was placed into a sterile plastic bag, overlaid with sterile 0.9% sodium chloride solution, and then bacterial cells were dislodged from the plant material by oscillation using a Pulsifier (Microgen Bioproducts LTD). A dilution series of the supernatant was spread on LB agar supplemented with the appropriate antibiotics and was incubated overnight at 28°C to calculate the colony forming units (CFU) g^{-1} of plant material.

In order to study the effect of *B. phytofirmans* PsJN and its mutants on the development of young plants, seeds of two maize varieties (Mazurka and Kaleo) were surface-sterilized and inoculated with bacteria as described above. Inoculated seeds

were planted in sterile magenta boxes filled with sterilized soil treated by three times freezing at -80°C and thawing. This treatment has been performed to eliminate insects and eggs of insects. The plants were grown for three weeks in the green house, after which the number of emerging plants, the length and the number of leaves were counted, and the fresh weights of the roots and the above ground plant parts were determined.

2.3 Results

2.3.1 Characterization of the AHLs produced by the QS systems of 3 different PBE *Burkholderia* spp.

In order to perform a complete analysis of which AHL molecules are produced by each QS system of the PBE *Burkholderia* spp., *braI* knockout mutants of *B. phymatum* STM815 and *B. phytofirmans* PsJN were constructed, yielding STM815BRAI and PSJNBRAI, respectively. As expected, TLC analysis using bacterial biosensors revealed that STM815BRAI was unable to produce any detectable AHL molecules, as the wild-type strain possesses only a single AHL QS system (BraI/R), whereas PSJNBRAI produced a putative OHC8-HSL molecule, which was most likely produced by the second AHL QS system, XenI2/R2, present in wild-type *B. phytofirmans* (Appendix Figure 7.2). Extractions of AHLs from these mutants and wild-type strains of *B. phymatum* and *B. phytofirmans* were performed, and these extracts were subjected to LC-ESI-MS/MS analysis, together with those from *B. xenovorans* LB400 and its QS mutants, LB400BRAI and LB400XENI2. These analyses showed that XenI2/R2 is mainly responsible for the production of OHC8-HSL, and that the BraI/R system is involved in the production of several different AHLs, varying in the length of their acyl chains (from 6 to 14 carbons) and in the substitution (or not) of a ketone or hydroxyl at position C3 (Table 2.2 and Appendix Table 7.3). From this analysis it was also evident that *B. phymatum* STM815 produces greater amounts of AHLs than the other species under these growth conditions (Table 2.2 and Appendix Table 7.3).

2.3.2 The BraI/R QS of *B. phymatum* STM815 responds to several different AHL molecules

It has been established previously that the BraR proteins of *B. unamae* MTI-641, *B. kururiensis* M130 and *B. xenovorans* LB400 respond well to OC14-HSL (Suárez-Moreno et al., 2008; Suárez-Moreno et al., 2010). However, as we established here that the BraI/R of *B. phymatum* is responsible for the production of significant amounts of several different AHLs in addition to OC14-HSL,

Table 2.2. Identification of the different AHLs produced by the BraI/R system of *B. phymatum* STM815, *B. phytofirmas* PsJN and *B. xenovorans* LB400 and by the XenI2/R2 system of *B. xenovorans* LB400

Strain	AHLs						3-oxo-HSLs						3-hydroxy-HSLs					
	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}
STM815[a]	-	+	++	+++	++	++	-	++	+++	+++	+++	++	-	+	+++	+++	+++	++
STM815BRAI[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PsJN[b]	-	-	+	-	+	+	-	-	-	+	+	++	-	-	++	+	+	+++
PsJNBRAI[b]	-	-	+	-	-	-	-	-	-	-	-	-	-	-	++	+	-	-
LB400[b]	-	-	+	-	+	++	-	-	+	+	++	+++	-	-	++	+	++	+++
LB400BRAI[b]	-	-	+	-	-	-	-	-	-	-	-	-	-	-	++	+	-	-
LB400XENI2[b]	-	-	+	-	+	++	-	-	+	+	++	+++	-	-	+	+	++	+++

[a]AHL extraction from cell-free spent supernatant from a culture grown overnight in 20 ml of KB medium

[b]AHL extraction from cell-free spent supernatant from a culture grown overnight in 50 ml of KB medium

-, no production; +, relative abundance < 100,000; ++, 100,000 < relative abundance < 1,000,000; +++, relative abundance > 1,000,000

it was of interest to determine which is the cognate AHL for the $BraR_{phym}$. In order to determine which AHL(s) $BraR_{phym}$ best responded to, we cloned the promoter region of $braI_{phym}$ into the pMP220 reporter plasmid (Spaink et al., 1987), yielding $pMPbraI_p$, which was then introduced into the STM815 derivative *braI* mutant, STM815BRAI. The $braI_{phym}$ promoter activity was determined in the presence of exogenously added AHL molecules which were found to be most abundantly produced by strain STM815 (Table 2.2 and Appendix Table 7.3). Testing promoter activity in STM815BRAI ($pMPbraI_p$) upon addition of different AHLs in three different concentrations (1 μM, 100 nM and 10 nM) showed that the activity of the $braI_{phym}$ promoter was induced with all the AHLs tested, with the exception of C8-HSL (Figure 2.1). This result suggests that the $BraR_{phym}$ is more promiscuous than the other BraR proteins tested so far, as it is able to respond to several different AHL molecules, even at low concentrations.

2.3.3 Determination of the BraI/R regulon by transcriptome analysis

The BraI/R AHL QS system is very well conserved among all members of the PBE *Burkholderia* group thus it was of interest to study its regulon in two species. Microarray profiling of two PBE *Burkholderia* spp. originating from different environmental niches was performed, namely the legume symbiont *B. phymatum* STM815 and the soil isolate *B. xenovorans* LB400. RNA was extracted from the wild-type strains and their respective QS mutants, STM815BRAI and LB400BRAR, at the end of logarithmic phase from three biological replicates. A set of eight differentially expressed genes from each microarray experiment was then chosen for validation with SQ RT-PCR, which displayed comparable results with the microarray/transcriptomic data (Appendix Table 7.4).

2.3.3.1 The BraI/R regulon of *B. xenovorans* LB400

The BraI/R QS system of LB400 influenced the transcriptional levels of 347 genes distributed among its 3 chromosomes by 1.5-fold or more, representing approximately 4% of the protein-encoding genes in the strain. The system positively

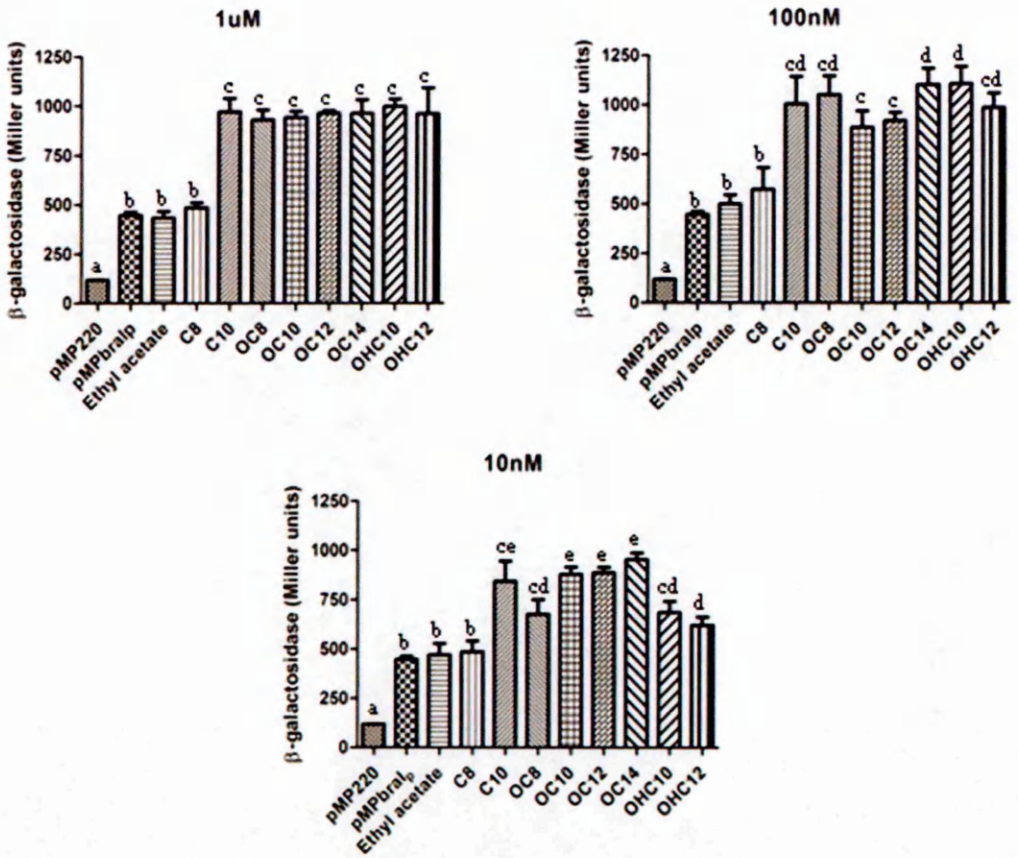

Figure 2.1. Determination of the biologically active AHL for the BraR AHL sensor/regulator of *B. phymatum* STM815. Bars correspond to β-galactosidase activities determined for STM815BRAI harboring pMP*braI*$_P$ or pMP220. Various exogenous AHLs in different concentrations (1μM, 100nM and 10nM) were added as indicated, and the β-galactosidase activities were determined. The results are mean values ± the standard deviations of three independent biological replicates. Means denoted by different letters are significantly different at $P \leq 0.05$

regulated 296 of these genes, whereas 61 were down-regulated (Appendix Table 7.5 and Figure 2.2).

The highest percentage of the regulated genes corresponded to cell process and metabolism genes (42.4%), and the second most represented set of genes encode for hypothetical proteins (27.1%) (Figure 2.2). A closer analysis of these hypothetical proteins with the STRING tool (Snel et al., 2000) showed that most were

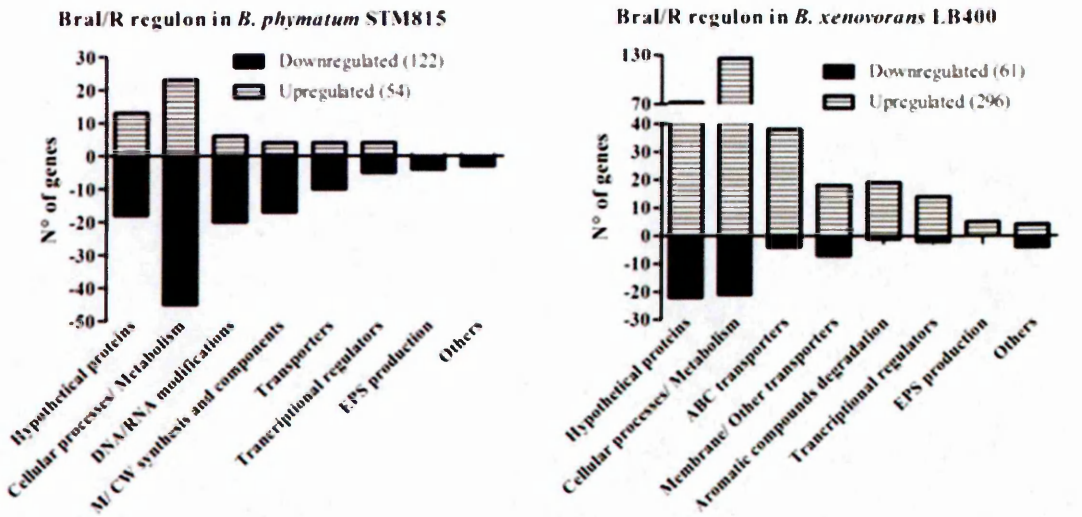

Figure 2.2. Functional classification of differentially expressed genes found in transcriptome analysis between *B. phymatum* STM815 wild-type and its *braI* mutant and *B. xenovorans* LB400 wild-type and its *braR* mutant. Only genes with a fold difference greater than 1.5 are included. M, membrane; CW, cell wall; EPS, exopolysaccharide.

conserved among bacteria, also having homologues in such distant groups as eukaryotes and Archaea. However, some of them were shared only with other *Burkholderia* spp. as for example loci Bxe_A2661, Bxe_B0810, Bxe_B2781 and others were unique to *B. xenovorans*, such as Bxe_B0044, Bxe_B0045 and Bxe_B0046 (see Appendix Table 7.5).

A significant finding was the over-representation of genes encoding components of ABC transporters, representing 12.1% of all differentially expressed genes (Figure 2.2). This finding suggests that QS is important for the interaction of LB400 with the environment, as ABC transporters are essential for the utilization of environmental nutrients. Moreover, 5.7% of the genes shown to be regulated by QS were involved in the degradation of aromatic compounds, a well-known characteristic of *B. xenovorans*, and several of them were related to benzoate degradation (Appendix Table 7.5).

2.3.3.2 The BraI/R regulon of *B. phymatum* STM815

The BraI/R QS system of *B. phymatum* STM815 acted directly or indirectly on the transcription of 176 genes by 1.5-fold or more, which represents approximately 2.3% of the protein-coding genes in this strain. The system positively regulated 54 of these genes, whereas 122 were down-regulated (Appendix Table 7.6 and Figure 2.2).

As in *B. xenovorans* LB400, the highest percentage of BraI/R-regulated genes (38.6%) corresponded to various cellular processes and metabolism genes. Again, hypothetical proteins were highly represented, with 17.6% of the total genes regulated above 1.5-fold (Figure 2.2). Of these, Bphy_1208 and Bphy_1217 were found only in *Burkholderia* spp., and Bphy_1241 was unique to STM815 (see Appendix Table 7.6).

A significant amount (14.8%) of the regulated genes was shown to be involved in DNA/RNA modifications, especially in recombination and DNA repair; in addition, 11.9% were involved in the synthesis and composition of the bacterial membrane and cell wall, with a good representation of genes related to the production of lipopolysaccharides (LPS). No genes for nodulation or nitrogen fixation were found to be regulated by BraI/R.

2.3.3.3 Comparison of the BraI/R regulons of *B. xenovorans* LB400 and *B. phymatum* STM815

It was interesting to discover that the BraI/R system of STM815, which possesses one AHL QS system, was responsible for the regulation of a smaller number of genes (i.e. 50% less) when compared to the BraI/R regulon of LB400, which also possesses an additional AHL QS system. Another important difference was the major type of regulation in each strain; in LB400 85.3% of the genes were activated by the system, whereas in STM815 69.3% were being repressed by it.

This comparison surprisingly revealed that only 4 genes were regulated in both strains (Table 2.3); these coded for a hypothetical protein, a peptidyl-tRNA hydrolase, and the other two were related to the production of EPS. Little can be predicted about the hypothetical protein, as it does not have any known conserved domains, but a BLAST search revealed that it is a very common protein among *Burkholderia* spp. Peptidyl-tRNA hydrolases are important for protein translation, as they release tRNA

Table 2.3. Homologous genes regulated by the BraI/R QS system in both *B. xenovorans* LB400 and *B. phymatum* STM815

Gene ID[a]	Gene product	Degree of identity	Fold change
Bphy_0422	Conserved hypothetical protein	89%	1.61
Bxe_A0642	Conserved hypothetical protein		1.64
Bphy_0313	Peptidyl-tRNA hydrolase	92%	-3.01
Bxe_A4135	Peptidyl-tRNA hydrolase		-1.56
Bphy_1058	Nucleotide sugar dehydrogenase	86%	-4.10
Bxe_A2245	UDP-glucose 6-dehydrogenase		2.48
Bphy_1064	Putative transmembrane protein	80%	-2.26
Bxe_A2239	Putative transmembrane protein		1.64

[a]Gene ID starting with Bphy, coming from *B. phymatum* STM815; Gene ID starting with Bxe, coming from *B. xenovorans* LB400.

from peptidyl-tRNA by cleaving the ester bond between the peptide and the tRNA, and they have been shown to be essential for the survival of *E. coli* (Singh and Varshney, 2004; Das and Varshney, 2006). The two EPS-related genes are part of an EPS production cluster, known as *bce-1*, composed of 11 genes homologous to the ones responsible for the synthesis of cepacian, the major EPS produced by a large percentage of clinical isolates of the BCC (Ferreira et al., 2010). Besides these two EPS genes that are being regulated in both strains, more genes from the same cluster were regulated by the BraI/R system in each bacterium, 2 other genes in STM815 and 3 in LB400 (Figure 2.3). However, the type of regulation of these EPS genes is different in each strain, as they are repressed by the QS system in STM815 and activated by it in LB400.

2.3.4 Confirmation of the regulation of EPS production by promoter studies and EPS quantification

Careful analysis of the promoter region of the *bce-1* of LB400 and STM815 did not reveal any evident BraR putative regulatory region via a lux-box-like element. We therefore cloned the promoter region of one of the *eps* genes (*i.e.* Bphy_1057, undecaprenyl-phosphate glucose phosphotransferase) of *B. phymatum* STM815 into the

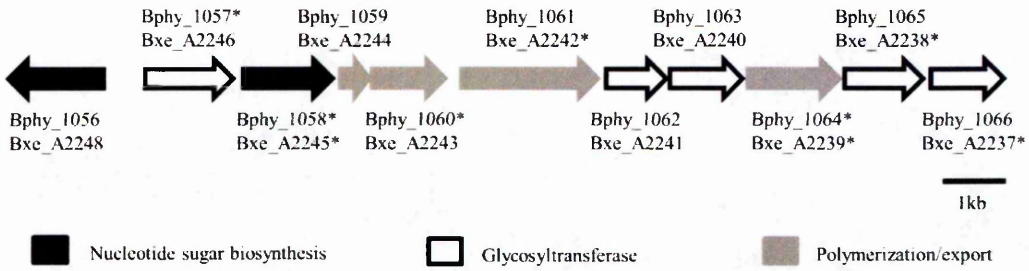

Figure 2.3. The cepacian production cluster *bce-I* from *B. xenovorans* LB400 and *B. phymatum* STM815. *, genes found to be regulated by the BraI/R QS system of LB400 (Bxe) or STM815 (Bphy) by microarray studies.

pMP220 reporter plasmid (Spaink et al., 1987), yielding the plasmid pMPeps$_p$, which was then introduced into the STM815 wild-type strain and its derivative *braI* mutant. β-galactosidase activity was determined at different growth stages and it confirmed the negative regulation of the EPS promoter by BraI/R (Figure 2.4). Promoter activity increased approximately 1.5-fold in the *braI* mutant throughout the growth phase and was restored to statistically similar levels by complementation with exogenous addition of AHLs.

We also quantified EPS from the wild-type and QS mutant strains of STM815 and LB400. Moreover, we performed the same experiment with the strains *B. phytofirmans* PsJN and *B. phymatum* GR01 and with their respective *braI* mutants in order to elucidate if BraI/R was involved in the regulation of EPS production also in these strains. *B. phymatum* STM815 and GR01 showed that their QS mutants produced substantially higher amounts of EPS (9.5- and 240-fold increase, respectively) than the the wild-type strains (Figure 2.5). In contrast, the QS mutants of *B. xenovorans* LB400 and *B. phytofirmans* PsJN produced less EPS than the wild-type strains, with a difference of 1.5- and 2.9-fold, respectively (Figure 2.5). In all four cases, EPS production was completely or partially restored to the wild-type levels when mutants were complemented with plasmids harbouring their corresponding *braI* or *braR* genes, as appropriate. These results were in agreement with the microarray and gene promoter data, and further confirmed the role of the BraI/R QS system in the regulation of EPS production in different PBE *Burkholderia* spp.

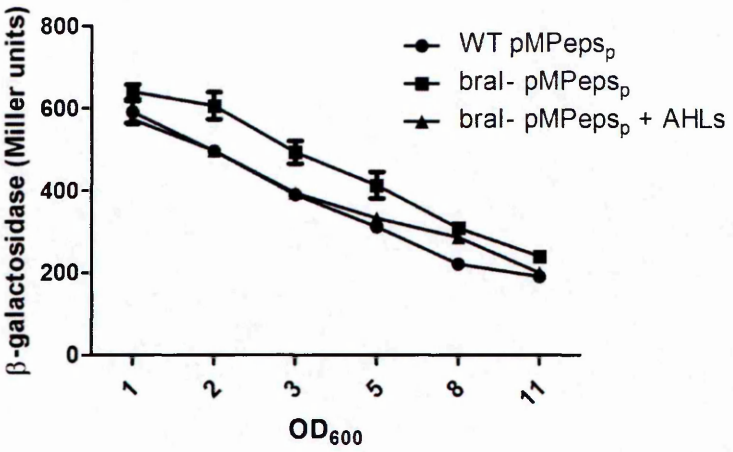

Figure 2.4. Transcription of the EPS operon is negatively regulated by the BraI/R QS system of *B. phymatum* STM815. ß-galactosidase activities of a transcriptional fusion of the EPS operon promoter region to *lacZ* were assayed in the *B. phymatum* STM815 wild-type (WT $pMPeps_p$), its QS mutant (braI- $pMPeps_p$), and the QS mutant complemented by the addition of 2 µM AHL to the growth medium (braI- $pMPeps_p$ + AHLs). Strains were grown in KB medium. The results are mean values ± the standard deviations of three independent biological replicates.

2.3.5 The regulation of EPS production by the BraI/R QS system is a common feature among the PBE *Burkholderia* group.

Our results have shown the involvement of BraI/R in the regulation of EPS production in *B. phymatum, B. xenovorans, B. phytofirmans, B. kururiensis* and *B. unamae* [see above; (Suárez-Moreno et al., 2010)]. These observations suggest a possible common role for the BraI/R system among the PBE *Burkholderia* group. To test this we mobilized the plasmid pME6863 (Reimmann et al., 2002), harbouring the gene coding for the AiiA lactonase enzyme, into five other PBE species, namely *B. tuberum* DSM17489, *B. tropica* Ppe8, *B. terrae* DSM17804, *B. phenazinium* DSM10684 and *B. graminis* DSM17151. We initially confirmed that the presence of pME6863 significantly reduced AHL production by AHL extraction and analysis with a biosensor strain (Appendix Figure 7.3). EPS production was then tested on solid growth media and all five *Burkholderia* strains carrying the pME6863 plasmid displayed a

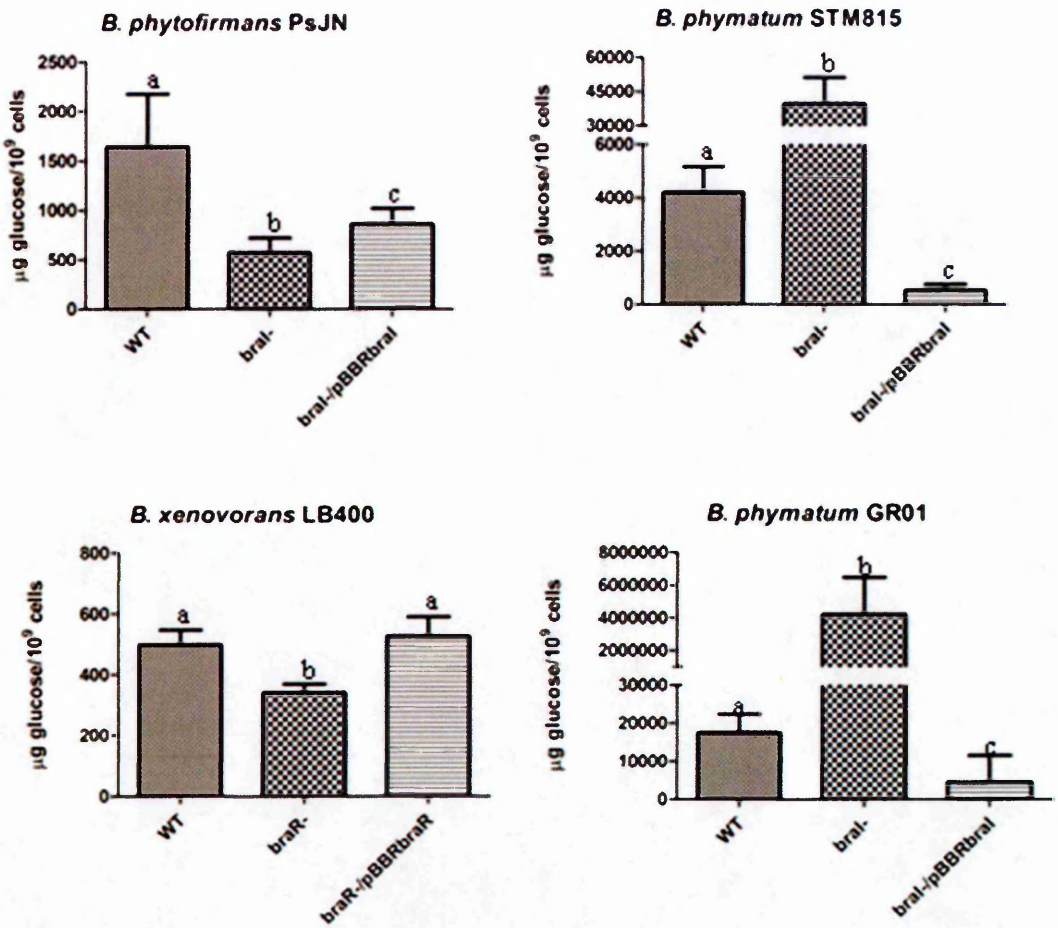

Figure 2.5. EPS quantification of *B. phytofirmans* PsJN, *B. phymatum* STM815, *B. xenovorans* LB400 and *B. phymatum* GR01 wild-type (WT), QS mutants (braI- or braR-) and complemented mutants (braI-/pBBRbraI or braR-/pBBRbraR). Experimets performed by the boiling phenol method (described in Materials and Methods) in triplicate, and means ± standard deviations are plotted. Means denoted by different letters are significantly different at $P \leq 0.05$

different profile of EPS production when compared to the wild-type strains (Appendix Figure 7.4). These results demonstrated that the conserved BraI/R AHL QS system is involved in the regulation of EPS production in the PBE *Burkholderia* group.

2.3.6 The BraI/R QS system is not essential for legume nodulation by *B. phymatum* spp.

It was of interest to determine the role of the BraI/R of *B. phymatum* in symbiotic nodulation. We therefore tested the ability of *braI* mutants of *B. phymatum* STM815 and GR01 to produce effective nodules on mimosa (*Mimosa pudica)* and common bean (*Phaseolus vulgaris)*, respectively.

Results showed that *M. pudica* was nodulated by the wild-type strain of STM815 and by the QS mutant strain to the same extent, as no difference in number of nodules was observed (Figure 2.6). In both cases the nodules were effective, as the dry weights of the inoculated plants were greater when compared to the un-inoculated (and therefore non-nodulated) controls (Figure 2.6). Similar results were obtained when using *B. phymatum* GR01 strain in nodulation experiments with common bean. The *braI* mutant GR01BRAI was as competent as the wild-type in producing symbiotic nodules on *P. vulgaris* var. Flamingo and var. Negro Jamapa (Appendix Table 7.7). Moreover, the dry weights of plants inoculated with the QS mutant were similar to those of plants inoculated with the wild-type strain (Appendix Table 7.8). These results clearly indicate that the BraI/R QS system of *B. phymatum* does not play a major role in the symbiotic lifestyle of this species.

2.3.7 The BraI/R QS system is not important for the endophytic colonization and plant growth promotion of maize by *B. phytofirmans* PsJN

B. phytofirmans PsJN colonizes endophytically and promotes the growth of several plant species, including maize (*Zea mays*), and so the latter was used as a model to study the role of BraI/R in endophytic colonization. Although PsJN colonizes and interacts with a wide variety of genetically unrelated plants, the intensity of its effects may vary considerably between different genotypes of a plant species (Trognitz et al., 2009; Da et al., 2012). For this reason, we performed experiments using two different maize cultivars (cv. Kaleo and cv. Mazurka).

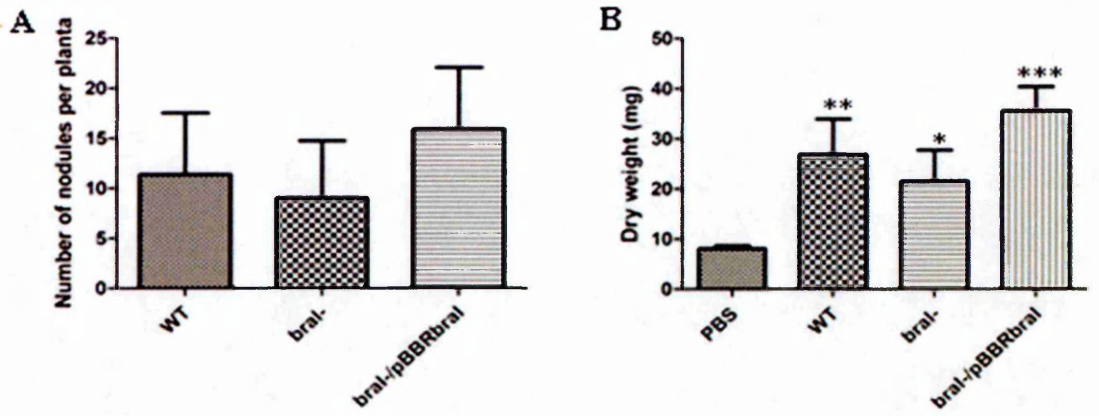

Figure 2.6. Nodulation of *M. pudica* by *B. phymatum* STM815 (WT), its QS mutant (braI-) or the complemented QS mutant (braI-/pBBRbraI) measured 60 days after infection. A, mean number of nodules per plant. B, mean dry weight of plants inoculated with the three different strains or left uninoculated (PBS). Nodulation tests were repeated twice, with 8 to 10 replicate plants. Bars indicate ± standard deviation. Statistical analyses (Student's *t* test) were performed to compare the mutant to the wild-type strain (A) or inoculated plants to the uninoculated ones (B). *, significant difference at $P \leq 0.05$; **, significant difference being $P \leq 0.01$; ***, significant difference being $P \leq 0.001$

PsJN colonized both maize varieties equally well, as we recovered a similar number of viable cells from four day-old seedlings (1.3E+08 $CFUg^{-1}$ fresh weight [FW] in cv. Mazurka and 1.4E+08 $CFUg^{-1}$ FW in cv. Kaleo). The PSJNBRAI mutant strain was not affected in its colonization ability of either maize variety, as the number of viable cells recovered was comparable to the wild-type strain (1.2E+08 $CFUg^{-1}$ FW in cv. Mazurka and 7.6E+07 $CFUg^{-1}$ FW in cv. Kaleo).

Germination was not significantly affected by PsJN wild-type strain in both maize varieties. By four days after seed inoculation no significant differences were observed in the number of germinated seeds and roots. Seedling root and shoot lengths were increased, but not significantly with respect to uninoculated seeds treated with broth only. Inoculation with the PSJNBRAI mutant strain gave similar results to the wild-type strain and to the uninoculated controls. In addition, PsJN did not significantly affect the growth of young plants of cv. Mazurka. Three weeks after seed inoculation

and growth in soil in the green house, no significant differences were observed in the number of leaves, length of sprouts and roots nor sprout fresh weight, with respect to uninoculated control plants. No effect was observed in the plants inoculated with the PSJNBRAI mutant strain. In contrast to cv. Mazurka, PsJN did show significant effects on the development of young plants of cv. Kaleo. Sprout length was increased from an average value of 18 cm in the control to 43 cm in PsJN inoculated plants, while the average root fresh weight was increased from 0.3 to 1.0 g plant^{-1}, and the average sprout weight increased from 0.7 to 1.7 g plant^{-1}. The PSJNBRAI mutant strain had similar effects on young plants of cv. Kaleo, thus showing that the *braI* knockout mutation did not affect the plant growth stimulating effect of the endophyte. Taken together, these results demonstrate that the BraI/R QS system of *B. phytofirmans* PsJN is neither involved in endophytic colonization nor in plant growth promotion of maize.

2.4 Discussion

The recently described PBE *Burkholderia* group is attracting attention from the scientific community as it is composed of metabolically versatile bacteria with great agrobiotechnological potential. This group shares a highly conserved AHL QS system, known as BraI/R, which in *B. kururiensis*, *B. unamae* and *B. xenovorans* was shown to produce and respond to OC14-HSL (Suárez-Moreno et al., 2010). In this study we present LC-ESI-MS/MS analyses of AHL extracts from members of this group (*i.e. B. phymatum* STM815, *B. phytofirmans* PsJN, *B. xenovorans* LB400 and their AHL QS mutants). We have identified and compared the regulons of BraI/R QS of *B. phymatum* STM815 and *B. xenovorans* LB400, which revealed that the regulons of this QS system are species-specific, which may be a result of niche adaptation. Moreover, this QS system was shown not to be important for legume nodulation by *B. phymatum* spp. nor for endophytic colonization and growth promotion of maize by *B. phytofirmans* PsJN.

The BraI/R system is responsible for the production of AHL molecules varying from 6 to 14 carbons with or without oxo or hydroxyl substitutions at the C3 position (Table 2.2 and Appendix Table 7.3). Some other LuxI-type proteins were already reported to produce several different types of AHL molecules. For instance, the AHL autoinducer synthases of some *Rhizobium* spp. are known to produce many different AHLs, as well as AfeI from *Acidithiobacillus ferrooxidans* (González and Marketon, 2003; Farah et al., 2005). LuxI-type proteins catalyze AHL synthesis from *S*-adenosylmethionine (SAM) and acylated acyl carrier protein (acyl-ACP). It has been proposed that the production of different types of AHLs is not only a function of the enzyme acyl chain specificity, but may also be influenced by the available cellular pool of acyl-ACP substrates in each bacterium (Watson et al., 2002). Consequently, growth conditions could also affect the acyl-ACP availability (Brader et al., 2005). This could explain why we observed that some AHL molecules were only produced in M9-glucose medium and not in KB or *vice-versa e.g. B. xenovorans* LB400 and *B. phytofirmans* PsJN were only able to produce C6-HSL when grown in M9-glucose (Appendix Table 7.3).

B. phymatum STM815 was shown to produce much higher amounts of each AHL molecule than *B. xenovorans* LB400 and *B. phytofirmans* PsJN (Table 2.2 and Appendix Table 7.3). Moreover, the $BraR_{phym}$ is able to respond to almost all of the

AHLs that it produces, even in concentrations as low as 10 nM, indicating that $BraR_{phym}$ has a broader signal specificity than the other BraR molecules already tested (Figure 2.1) (Suárez-Moreno et al., 2010). The high production and promiscuous response to different types of AHLs by BraR even at low concentrations could mean that *B. phymatum* is an efficient eavesdropper of AHLs produced by neighbouring bacteria, and possibly provides it with a competitive advantage (Chandler et al., 2012).

The presence of the BraI/R QS system among all the species of the PBE group tested so far possibly suggests that it was originally present in their common ancestor and is part of their core genome (Suárez-Moreno et al., 2010). Does BraI/R therefore share a common regulon? For this purpose we performed transcriptome analysis of two PBE *Burkholderia* spp. from different environmental niches, namely the legume symbiont *B. phymatum* STM815 and the soil-borne, efficient biodegrader, *B. xenovorans* LB400. The regulon of *B. xenovorans* is composed of several hypothetical proteins (27.1%) from which 10 are unique to the species, and thus could be a topic of future research. Interestingly, 12.1% of all the genes being regulated encode ABC transporter components, which are important for the transport of a variety of substrates, such as metals, small ions, mono- and oligosaccharides, peptides and amino acids, being essential for the utilization of environmentally available nutrients (Giuliani et al., 2011). Moreover, an important amount of genes involved in the degradation of aromatic compounds, especially benzoate, were also regulated byBraI/R. It is possible that these two functions are linked, as ABC transporters are known to be involved in the uptake of aromatic compounds (Giuliani et al., 2011).

Aromatic compounds are widely distributed throughout the biosphere, predominantly in the form of recycled plant material, and comprise >25% of the earth's biomass (Gibson and Harwood, 2002). The natural turnover of these molecules is very slow because of the inherent thermodynamic stability of the aromatic ring. For this reason, microbial biodegradative pathways play a crucial role in the carbon cycle of aromatic compounds. Benzoate is a key aromatic intermediate in these pathways, being an end product of the degradation of many aromatic compounds, such as toluene, phenol, and even polichlorobiphenyl. *B. xenovorans* possesses three functional benzoate degradation pathways in which two are paralogous copies of the benzoate oxidation (box) pathway (box_C - chromosomal and box_M - megaplasmid) (Chain et al., 2006;

Denef et al., 2006). Our studies revealed regulation of the box_M cluster by the BraI/R system, which is also in accordance with previous studies in which it was shown that the expression of this pathway is activated during the transition to the stationary phase of growth (Denef et al., 2004; Denef et al., 2005).

In contrast to *B. xenovorans* LB400, the regulon of BraI/R of *B. phymatum* was over-represented in loci involved in the regulation of DNA/RNA modification enzymes, in DNA repair and recombination, and in the regulation of components of the membrane/cell wall or of enzymes important for their synthesis. The negative regulation of genes related to LPS production is an interesting finding, as previous studies had already shown that the rhizobial LPS undergoes structural modifications during symbiosis with legumes (Brewin et al., 1986; Tao et al., 1992; Kannenberg and Carlson, 2001; Noel et al., 2004; D'Haeze et al., 2007). LPS is the primary component of the bacterial outer layer and is comprised of three structural regions: the lipid A that is anchored in the bacterial outer membrane, the core oligosaccharide, and the O-chain polysaccharide (or O antigen), which may or may not be present. Studies with α-rhizobia have shown that there are compositional differences between the bacterial and bacteroid LPSs (Brewin et al., 1986; Tao et al., 1992; Kannenberg and Carlson, 2001; Noel et al., 2004; D'Haeze et al., 2007), and this has led to the hypothesis that the bacterial LPS structure inside legume root nodules is probably controlled, to a large extent, by the *in planta* micro-environment. From our studies, it cannot be excluded that AHL QS might play a role in LPS biosynthesis and modification during legume nodulation by *B. phymatum*; no studies thus far correlating LPS production/modification and nodule formation have been reported for β-rhizobia.

The regulon of *B. phymatum* was determined using a *braI* mutant whereas for *B. xenovorans* we used a *braR* mutant; this could have generated slight differences, as BraR could be involved in regulating gene(s) independently of AHLs. Moreover, *B. xenovorans* contains a LuxR solo which could respond to BraI produced AHLs expanding the QS regulon. As AHL QS systems strictly require both LuxI and LuxR-like proteins, we believe that our results using two different mutants of two species can be reliably compared. Moreover, our analysis provides a snapshot of the quorum-controlled regulons at only one time-point in the growth phase of these bacteria. This approach certainly has limitations and cannot provide an exhaustive census of quorum-

controlled genes. Nevertheless, it indicates a unique role for BraI/R in each species, rather than a common one as only four common genes were being regulated in two species of the PBE cluster. Interestingly, 2 of the 4 genes commonly regulated by both BraI/R systems are involved in the production of the EPS cepacian (Figure 2.3). The regulation of EPS genes in *B. phymatum* and *B. xenovorans* was also confirmed by gene promoter and phenotypic studies. The regulation of EPS in several other members of the PBE *Burkholderia* group was also established by the introduction of an AHL-lactonase gene. The regulation of EPS production by AHL QS is, therefore, a common feature in PBE *Burkholderia* species. Exopolysaccharides are abundant extracellular products that accumulate on the bacterial cell surface and are secreted into its surroundings. The formation of a highly polymerized, hydrated, anionic matrix with a peripheral localization suggests that EPS protects bacteria against various environmental stresses, such as desiccation or toxic molecules, and it can also provide the first contact between bacteria and plant cell surfaces (D'Haeze et al., 2004). These factors make the production of EPS an important phenotype for both plant and soil bacteria, and might, be a reason why it is regulated by the BraI/R in the PBE *Burkholderia* spp. It would be interesting to perform further transcriptomic analyses with PBE species that occupy similar niches in order to provide further insights into the evolution of the role of the BraI/R system.

Although several studies with α-rhizobia have shown the importance of LPS and EPS in their symbiotic interactions with legumes (Gibson et al., 2008), the *braI* mutants of *B. phymatum* were, surprisingly, not affected in their ability to form nodules on *M. pudica* and *P. vulgaris*. However, all these α-rhizobial studies were performed with mutants impaired in the production of LPS or EPS, while the *braI* mutants of *B. phymatum* are probably producing more of these molecules, as our transcriptome studies have shown that the BraI/R QS system is negatively regulating LPS and EPS genes. The AHL QS systems of several α-rhizobia have been shown to play important roles in legume nodulation and nitrogen fixation (González and Marketon, 2003; Downie, 2010). Our study represents the first analysis of the role of QS in nodulation by β-rhizobia and it has revealed, somewhat surprisingly, that AHL QS does not play a major role in it, and we are currently undertaking further research into this phenomenon.

A recent study demonstrated the importance of the XenI2/R2 QS system of *B. phytofirmans* PsJN for its endophytic colonization and growth promotion of *Arabidopsis thaliana*, but a PsJN knockout mutant for the BraI/R system only showed reduced endophytic colonization when compared to the wild-type (Zúñiga et al., 2013). In the present study, however, we showed that the BraI/R QS system of PsJN does not play a major role in its endophytic colonization and plant growth promotion of maize. This contrasts with studies on rice, in which the BraI/R AHL QS system of *B. kururiensis* M130 has been shown to be important in its endophytic colonization (Suárez-Moreno et al., 2010). It is possible that different endophytes use different regulatory processes in order to colonize their hosts, with the plant also playing an active role in the colonization process (Rosenblueth and Martínez-Romero, 2006).

It is important to highlight that these *Burkholderia* spp. were found to possess another type of QS system, based on the production and recognition of a molecule known as the *Burkholderia* diffusible signal factor (BDSF) (Deng et al., 2012). This system was shown to regulate swarming motility, biofilm formation and virulence in *B. cenocepacia* (Deng et al., 2012) and it could be involved in plant-bacteria interaction in the PBE *Burkholderia* spp.

In summary, this study highlighted that regardless of how well conserved BraI/R is among the PBE *Burkholderia*, the regulon is not conserved, possibly indicating that its role has evolved to be tailored to different models of growth in the various niches that this cluster of *Burkholderia* species occupies. Surprisingly, BraI/R does not play a major role in these *Burkholderia in planta*, which means that other regulatory systems, possibly both global and specific, are regulating genes in response to the *in planta* growth of these bacteria. The production of EPS, however, is regulated by the BraI/R system throughout the PBE *Burkholderia* cluster, thus indicating a common role of EPS at high densities in this group of bacteria. Future studies will need to focus on the direct targets as well as the role played by the second AHL QS system that some species in this PBE *Burkholderia* cluster have evolved to possess.

3 Genome sequencing and analysis of the *Bukholderia kururiensis* M130 genome reveals many potential loci involved in the endophytic lifestyle

3.1 Introduction

It is predicted that rice (*Oryza sativa*) production needs to increase by 60% over the next 40 years (FAO, 2012). Limiting factors in production are in part due to the effects of biotic (*e.g.* viral, bacterial and fungal diseases) and abiotic stress factors (environmental stresses like temperature, drought and lack of available nutrients) which seriously limit its growth and yields. Efficient fertilizer used together with adequate pest management practices are required in order to improve and sustain productivity of rice worldwide. The use of bioinoculants as a replacement to conventional chemical fertilizers is one of the modern tools for agriculture and their utilization is significantly increasing. Bioinoculants mainly consist of microorganisms which can promote the supply of nutrients (converting complex nutrients to simple and plant-available ones) to the host plants and/or assist the plant in the control of pathogens. There are different types of microorganisms which are used as biofertilizers, many of which colonize the roots of the host plant. Endophytes are recently attracting considerable interest as they are microorganisms that spend the entire or part of their life cycle living inside the plant without causing any harm (Reinhold-Hurek and Hurek, 1998; Compant et al., 2010; Reinhold-Hurek and Hurek, 2011). Although the interaction between endophytic bacteria and host plants is currently not understood, it is well established that some of these associations are beneficial to the plant (Reinhold-Hurek and Hurek, 1998, 2011). Endophytes are therefore gaining commercial interest due to their potential to improve plant growth and to the possible advantages in their application. Possible mechanisms for improvement of plant growth by endophytes include nitrogen fixation, phosphate solubilization, iron chelation and production of plant growth hormones and anti-microbial agents (Hardoim et al., 2008; Berg, 2009).

Burkholderia kururiensis M130, formerly known as "*Burkholderia brasilensis*", is an endophyte isolated from rice in Brazil and is to able promote plant growth at least in part by increasing nitrogen availability to the rice plant through biological nitrogen fixation (Baldani et al., 1997b; Baldani et al., 2000). The inoculation of rice seeds with this strain, under gnotobiotic conditions revealed that *B. kururiensis* M130 can fix up to 30% of the total nitrogen accumulated increasing rice yield and growth (Baldani et al., 2000). Here we present the genome sequence of *B. kururiensis* M130 which has been

annotated and analysed in order to further characterize and identify potential mechanisms involved in its beneficial interaction with the plant.

3.2 Materials and Methods

3.2.1 DNA extraction, sequencing and annotation

Total DNA from *B. kururiensis* M130 was isolated by sarkosyl-pronase lysis as described previously (Better et al., 1983) from an overnight culture grown at 30°C in King's medium (KB) (King et al., 1954). An Illumina (Bennett, 2004) GAII shotgun library (3,320,000 reads totaling 332.0 Mb) and a paired-end 454 (Margulies et al., 2005) GS-FLX library (445,966 reads totaling 163.7 Mb) were generated and sequenced. *De novo* assembly was performed using MIRA (Mimicking Intelligent Read Assembly) version 3.4.0 (Chevreux, 2005) followed by manual curation. Automated annotation of the *B. kururiensis* M130 draft genome sequence was performed using RAST (Aziz et al., 2008).

3.2.2 Whole genome analysis of *B. kururiensis* M130

Analysis of *B. kururiensis* M130 genome sequence was performed in Unix environment with usage of Perl scripts and MUMmer (Kurtz et al., 2004). Categorization of putative coding protein sequences was done in RAST. The genome of *B. kururiensis* M130 was aligned against the one of *B. phenoliruptrix* BR3459a using the software Mauve 2.3.1 build 173 (Darling et al., 2010) to generate an image of the whole genome alignment.

Specific proteins were routinely identified using the BLASTP (e-value < 1e-10) function against *B. kururiensis* M130 genome in RAST webserver or, in some cases (*e.g.* proteins involved in the degradation of organic compounds and in the survival against plant defences) by clusters of orthologous groups (COG). Comparison between gene clusters and neighbourhood were performed using the IMG/ER server (Markowitz et al., 2012).

3.3 Results and Discussion

3.3.1 Genome sequencing and annotation

Genome sequencing was performed using a combination of Illumina and 454 technologies. Altogether, 3,961,173 pairs of reads were obtained with a ~60-fold coverage of the ~7.1 Mb genome. The *de novo* assembly followed by manual curation yielded 83 contigs organized in 9 scaffolds. The longest scaffold obtained was 1,922 kbp long. The genome of *B. kururiensis* M130 presents a G+C content of 63% and, according to the automated annotation done using RAST, contains a total of 6,568 predicted protein-coding genes from which 1,707 (26%) were annotated as encoding putative hypothetical proteins. A total of 63 RNA coding sequences were also identified in the RAST annotation.

The RAST server classified 3,240 predicted coding sequences into subsystems according to their putative function generating 27 different categories (Figure 3.1). The genes assigned to the subsystems of carbohydrate metabolism represent 24.6% of the total assigned loci of the strain. Genes related to the metabolism of aromatic compounds are also well-represented and comprise 5.2% of the assigned loci. Although *B. kururiensis* M130 is not recognized as a pathogenic strain, RAST assigned 155 genes to the category "virulence, disease and defense" (Figure 3.1). A closer look of this group however reveals that most of these genes are related to resistance to antibiotics and toxic compounds and production of bacteriocins (Figure 3.2). In addition, some loci of this group that were further assigned as "invasion and intracellular resistance" are highly conserved proteins such as ribosomal proteins, translation elongation factors, RNA polymerases which were shown to be essential for the growth of pathogenic *Mycobacterium tuberculosis* (Sassetti et al., 2003).

3.3.2 Whole-genome comparative analysis

Whole genome alignment against sequenced *Burkholderia* species indicated that *B. kururiensis* M130 genome is not highly similar to any other species of the genus (Table 3.1). This is not so surprising as it is known that the majority of the genomes of *Burkholderia* species have been acquired by horizontal gene transfer, a feature that

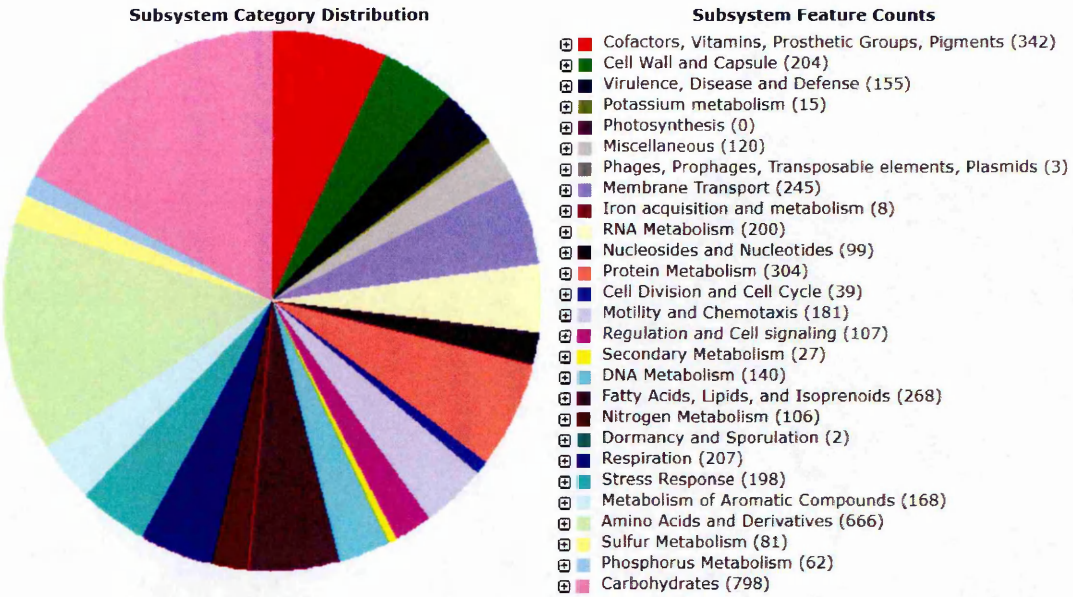

Figure 3.1. RAST subsystem features categorization. Total number of genes that were assigned to subsystems was 3240.

Figure 3.2. RAST subsystem "virulence, disease and defense". Number of genes of *B. kururiensis* M130 allocated in each subcategory is shown in parenthesis.

Table 3.1. Whole genome multiple genome alignment of B. kururiensis M130 using MUMmer.

Reference Alignment	Reference	Query Alignment[1]	Average identity
19.05 %	*Burkholderia unamae* MTI-641	25.6 %	85.34 %
24.73 %	*Burkholderia phenoliruptrix* BR3459a	23.6 %	85.06 %
20.15 %	*Burkholderia tuberum* STM678	23.36 %	85.01 %
19.42 %	*Burkholderia phytofirmans* PsJN	21.71 %	84.7 %
23.83 %	*Burkholderia phymatum* STM815	20.39 %	84.94 %
22.35 %	*Burkholderia* sp. KJ006	20.16 %	85.16 %
20.01 %	*Burkholderia vietnamiensis* G4	19.98 %	85.18 %
18.15%	*Burkholderia cenocepacia* J2315	19.68%	85.08%
18.92 %	*Burkholderia pseudomallei* 1026b	19.05 %	85.05 %
19.02 %	*Burkholderia glumae* BGR1	17.73 %	84.89 %
15.27 %	*Burkholderia gladioli* BSR3	17.14 %	84.73 %

[1] Query stands for M130 genome sequence

contributes to their genomic plasticity and niche versatility (Chain et al., 2006; Martinez-Aguilar et al., 2008).

To generate a more comprehensive comparison between *B. kururiensis* M130 genome and the closest complete sequenced species (*i.e. B. phenoliruptrix* BR3459a), an alignment using a different algorithm based on progressiveMauve was used. As observed in the results of MUMmer, multiple alignment percentage of M130 genome aligned against *B. phenoliruptrix* BR3459a was low, although the aligned regions usually possessed a high identity (Figure 3.3).

3.3.3 The *Burkholderia kururiensis* M130 genome and endophytic colonization

The genome of strain M130 was analyzed for possible determinants that could be important for the endophytic lifestyle including detoxification of toxic compounds, protein secretion, movement, adhesion and transporters. Below the classes of these determinants are presented and discussed.

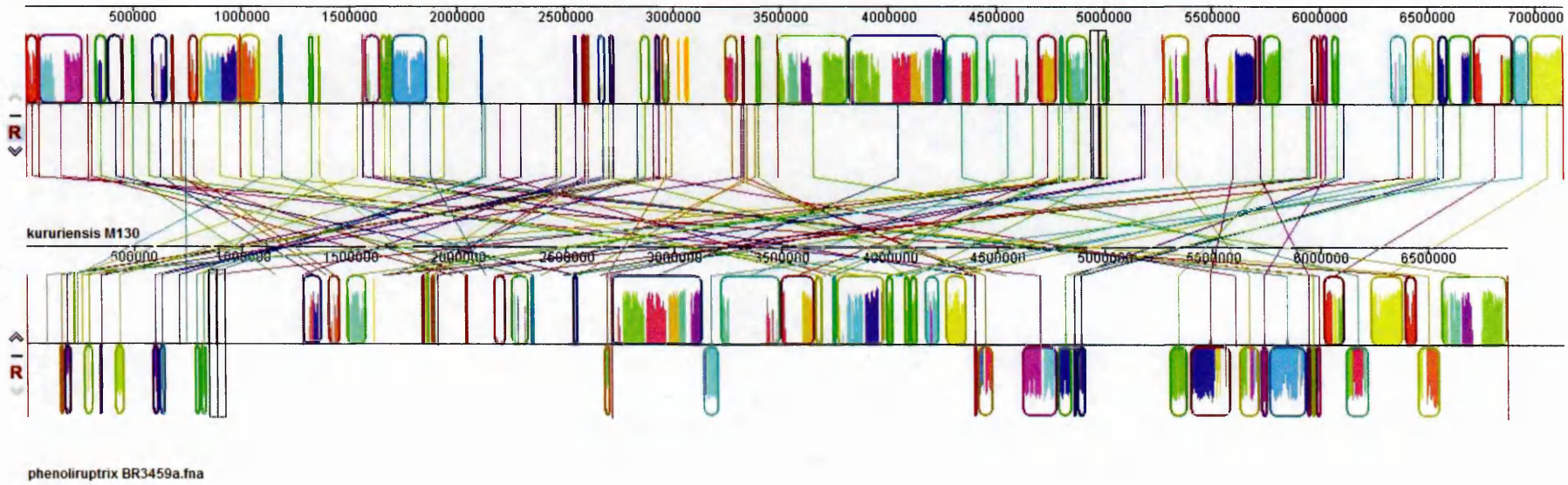

Figure 3.3. Whole genome alignment of *B. kururiensis* M130 and *B. phenoliruptrix* BR3459a using progressiveMauve aligner. Color levels inside each block represent identity level of nucleotide sequences present in respective regions.

3.3.3.1 Motility and root surface colonization

To successfully colonize the interior of the plant, endophytes must be able to reach the roots and form adherent microbial populations on the root surface (Hardoim et al., 2008). Flagella are not only involved in motility, but were also shown to be important for adherence, biofilm formation and host colonization (Merritt et al., 2007). The *B. kururiensis* M130 genome presents a large region with more than fifty genes predicted to encode features related to flagellar motility. Moreover, this genome possesses one gene for a hemagglutinin protein from the ShlA/HecA/FhaA family, five genes presenting a trimeric autotransporter adhesin-domain and several gene clusters involved in the production of type IVa (*pilA*, *pilBCD* and *pilMNPQ* localized in different parts of the genome) and IVb pili (three different gene clusters are present for the production of type IVb pili) (Figure 3.4). Little is known on the importance of type IV pili for the plant-endophyte interaction, however it was shown to be essential for endophytic rice colonization by *Azoarcus* sp. strain BH72 (Bohm et al., 2007), and has also been implicated in attachment to roots, twitching motility and biofilm formation (Dörr et al., 1998; O'Toole and Kolter, 1998b; Bohm et al., 2007).

3.3.3.2 Plant growth-promoting (PGP) traits

Previous studies have shown that rice plants inoculated with the endophyte *B. kururiensis* M130 resulted in 30% of the total nitrogen accumulated by rice plants coming from N-fixing activity of strain M130 (Baldani et al., 2000). As expected, genome sequencing performed here has demonstrated that a complete *nif*-region localized in contig 1 is present. This region is highly identical to the corresponding region of *B. unamae* MTI-641 (Figure 3.5), a closely related *Burkholderia* which is a maize endophyte.

Moreover, endophytes are usually able to produce plant hormones and other direct plant beneficial factors resulting in the promotion of plant growth (Hardoim et al., 2008). M130 contains the gene for 1-aminocyclopropane-1-carboxylate (ACC) deaminase that degrades the ethylene precursor ACC to 2-oxobutanoate and ammonia. Ethylene is a phytohormone involved in the modulation of plant growth and development as well as in its response to stress. Studies have shown that a sustained

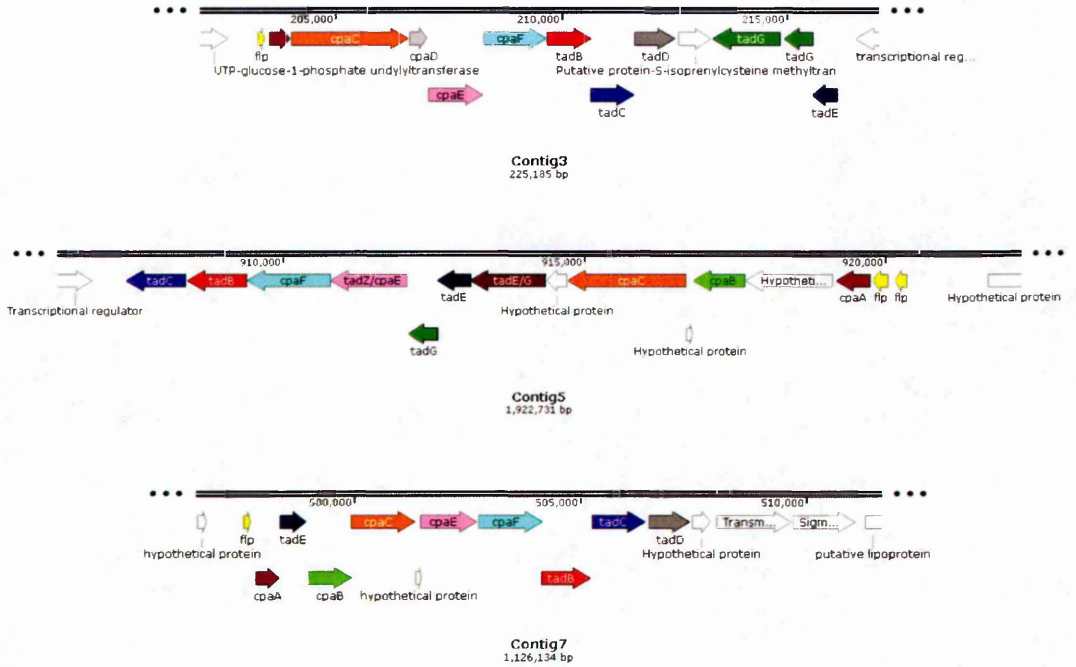

Figure 3.4. Genomic representation of the type IVb pili clusters of *B. kururiensis* M130. Genes of the same color (except white) are from the same COG family. Genes not known to be related to type IV pili production are assigned in white.

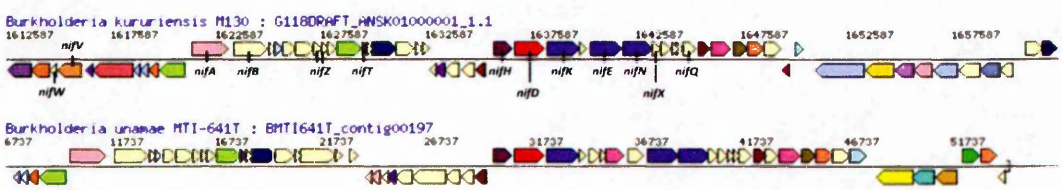

Figure 3.5. Genomic representation of the *nif*-region of *B. kururiensis* M130 and *B. unamae* MTI-641. Homologous of characterized *nif* genes have their names assigned. Genes of the same color (except white) are from the same orthologous group (top COG hit). White genes have no COG assignment.

high level of ethylene may inhibit root elongation, thus, ACC deaminase-producing bacteria can protect stressed plants from some of the deleterious effects of ethylene (Glick, 2005; Hardoim et al., 2008). It is now believed that plants select for endophytic

bacteria having ACC activity which would then constantly attenuate ethylene-derived stress (Hardoim et al., 2008).

Indole-3-acetic acid (IAA) is a phytohormone known for its involvement in physiological and developmental processes in plants, such as seedling and root growth and vascular patterning (Sukumar et al., 2013). Many bacterial strains are able to produce IAA improving plant growth and development (Hardoim et al., 2008; Sukumar et al., 2013). IAA synthesis proceeds via several pathways, which are partially present in strain M130 (Figure 3.6). In the indole-3-acetamide pathway, strain M130 possesses the *iaaH* gene encoding the hydrolase enzyme that produces NH_3 and IAA from indole-3-acetamide. However, the essential tryptophan 2-monooxygenase, encoded by *iaaM*, responsible for the decarboxylation of tryptophan to indole-3-acetamide was not unambiguously identified. Moreover, *B. kururiensis* M130 also lacks an *ipdC* homolog, which is essential for the indole-3-pyruvate pathway, where IAA is synthesized by indole-3-pyruvate decarboxylase from tryptophan via indole-3-pyruvic acid. However, M130 presents the last enzyme of the indole-3-acetonitrile pathway, which transforms indole-3-acetonitrile into IAA. Tryptophan-independent pathways where transferases transform indoles into IAA are present in the genome, however none of them encode a clear prototype of IAA-producing enzyme. No experiments have thus far been performed in order to evaluate the production of IAA by *B. kururiensis* M130, however studies showed that *B. kururiensis* strain KP23 is able to produce IAA (Mattos et al., 2008).

3.3.3.3 Survival against plant defenses and degradation of aromatic compounds

Plants are known to use several general ways to defend themselves against incoming microbial threats, amongst these are the production of reactive oxygen species (ROS) and nitric oxide (Hammond-Kosack and Jones, 1996; Zeidler et al., 2004). The genome of *B. kururiensis* M130 presents different features that can potentially allow strain M130 to overcome plant defenses. There are three superoxide dismutases, six putative catalases, an hydroperoxide reductases (encoded by two *ahpC* and one *ahpD*), two thiol peroxidases, one peroxiredoxin, one glutathione peroxidase, three other peroxidases, and twelve putative glutathione-S-transferase (GST) or GST

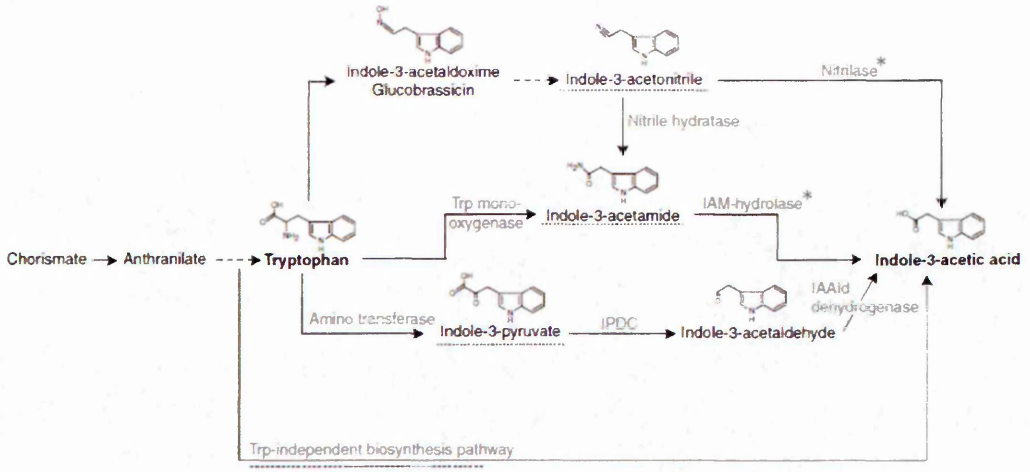

Figure 3.6. Overview of IAA biosynthetic pathways analyzed in *B. kururiensis* M130 genome. Dashed lines refer to the name of pathways. Enzymes unambiguously identified in *B. kururiensis* M130 are marked with an asterisk. IAAld, indole-3-acetaldehyde; IAM, indole-3-acetamide; IPDC, indole-3-pyruvate decarboxylase; Trp, tryptophan.

domain/family proteins that can defend the bacterial cell against ROS. Moreover, the presence of coding sequences specific for aerobic nitric oxide detoxification (flavohemoprotein) suggests that M130 possesses the ability to detoxify nitric oxide. Lastly, the genome of *B. kururiensis* M130 presents at least eighteen genes encoding RND (Resistance-Nodulation-Division) multidrug efflux pump components which could be involved in the removal of toxic molecules. In plant pathogenic *Erwinia amylovora* for example, RND pumps have been shown to be required for the export of apple tree phytoalexins, which are plant secondary metabolites with antimicrobial activity (Burse et al., 2004).

Plants produce many different organic aromatic compounds, thus it is expected that endophytes present features that enable them to degrade such metabolites for energy sources. *B. kururiensis* M130 carries genes coding various degradation enzymes, such as three alkane monooxygenase, which break-down aliphatic hydrocarbons. Moreover, at least 22 dioxygenase genes are present including ring cleavage enzymes such as catechol 1,2-dioxygenase, catechol 2,3-dioxygenase, and protocatechuate 3,4-

dioxygenase. These enzymes are required for the oxidation of aromatic compounds and allow bacteria to successfully colonize the plant interior by conferring both metabolic flexibility and protection against plant-derived toxic chemicals.

3.3.3.4 Protein secretion

The genome of *B. kururiensis* M130 encodes for the type II, III, V, VI, Sec-SRP (general secretory pathway) and the Tat (twin-arginine translocation) systems, but lacks a type IV secretion system, as shown in Figure 3.7. The presence of a T1SS in this strain is unclear, as only one gene of this system was found (*i.e.* outer membrane protein). Types III, IV and VI secretion systems have already been implicated in the delivery of proteins directly into the cytoplasm of eukaryotic cells by pathogenic bacteria (Tseng et al., 2009). However, in non-pathogenic bacteria, as the case of *B. kururiensis* M130, these systems might be involved in plant-bacteria interactions.

The T3SS of pathogenic bacteria is involved in the manipulation of host cellular immunity and metabolism in order to benefit the pathogen. However, this type of secretion system may also contribute to beneficial host-bacteria interactions as it was shown for *Bradyrhizobium elkanii* and *Rhizobium* sp. NGR234. During the interaction of these beneficial bacteria with their hosts, the T3SS is able to activate host nodulation signalling leading to symbiotic interactions (Kambara et al., 2009; Okazaki et al., 2013). In the case of *Rhizobium* sp. NGR234, the secreted effectors can either stimulate or block the nodulation process depending on the host legume, serving as a recognition mechanism (Kambara et al., 2009). Moreover, other endophytes have already shown to possess a T3SS such as *B. phytofirmas* PsJN (Mitter et al., 2013) and *Herbaspirillum seropedicae* SmR1 (Pedrosa et al., 2011), however no data is available on the possible role of this system during endophytic colonization.

T6SS genes are abundantly represented in metagenome studies of rice root endophytes suggesting that it is common among endophytes (Sessitsch et al., 2012). The T6SS is involved in bacteria-bacteria interactions as well as with eukaryotic hosts, both in pathogenic and in symbiotic relationships (Bingle et al., 2008; Cascales, 2008; Filloux et al., 2008; Basler et al., 2013). *Burkholderia pseudomallei*, an environmental saprophyte and the causative agent of melioidosis

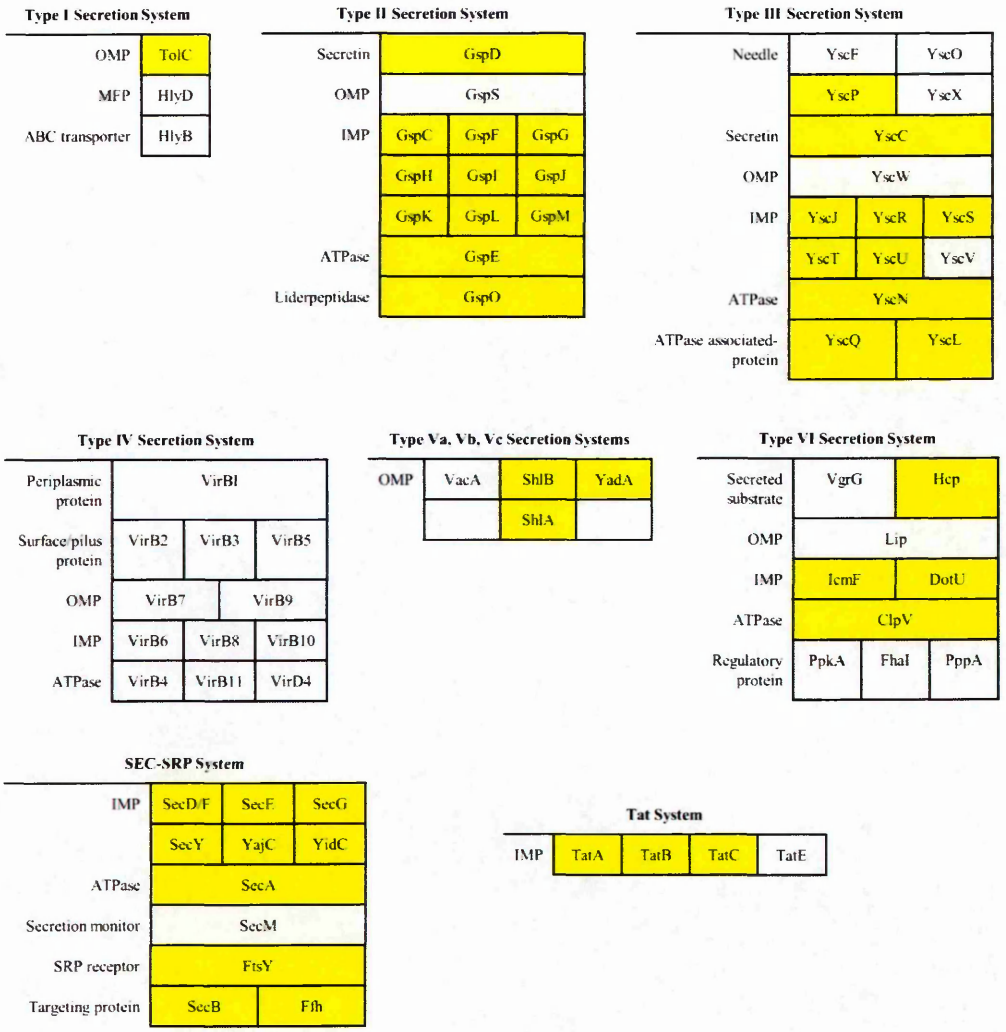

Figure 3.7. Presence of secretion systems in *B. kururiensis* M130. Genes indicated in yellow have homologs in strain M130. Missing genes are shown in white (modified from (KEGG, 2014).

possesses six evolutionarily distinct T6SSs (Schell et al., 2007). Studies suggest that the T6SS-5 of this bacterium, that is also shared among *Burkholderia thailandensis* and *Burkholderia mallei*, is the only system required for *Burkholderia* spp. pathogenesis to eukaryotic cells, both *in vitro* and *in vivo* (Pilatz et al., 2006; Schell et al., 2007; Schwarz et al., 2010). Moreover, the T6SS-5 clustered closely with eukaryotic cell-targeting systems, while the other T6SS systems clustered among the bacterial cell-targeting systems (Schwarz et al., 2010). The T6SS of *B. kururiensis* M130 has high

homology with the T6SS-1 of *B. pseudomallei* (Figure 3.8) with identity values ranging between 67 and 92%. This T6SS-1 locus of *B. pseudomallei* was shown to be important for interspecies bacterial interactions as mutants in this cluster were less fit to compete with other bacterial species than the wild-type strain (Schwarz et al., 2010). These results might indicate that also the T6SS of *B. kururiensis* M130 is involved in inter-bacterial, rather than host-bacteria interactions.

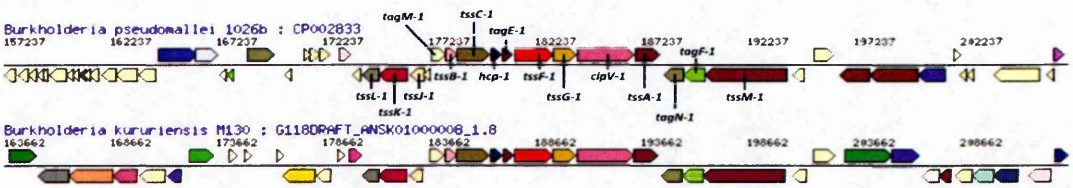

Figure 3.8. Genomic representation of the T6SS gene cluster of *B. kururiensis* M130 and *B. pseudomallei* 1026b. Genes were identified according to the nomenclature proposed by Shalom and colleagues (Shalom et al., 2007). Genes of the same color (except white) are from the same orthologous group (top COG hit). White genes have no COG assignment.

3.3.3.5 Genes involved in endophytic colonization in *Burkholderia* spp.

In order to gain a better understanding on the molecular basis of plant-endophyte interactions, there has recently been a great interest in identifying genes responsible for endophytic colonization. To date, no experimental evidence is present and only genome analysis has been performed indicating that a wide spectrum of putative loci are probably involved in endophytic colonization (Mitter et al., 2013). A recent genome analysis has identified a set of genes possibly responsible for endophytic behavior in the *Burkholderia* genus (Ali et al., 2014). This study revealed forty putative genes being involved in endophytism which have also been indicated in other genome studies and are mainly related to transporters, secretion and delivery systems, plant polymer degradation, transcriptional regulators and detoxification systems (Ali et al., 2014). The search for these genes in the genome of *B. kururiensis* M130 by BLASTP revealed that

all but three are present (Table 3.2). This result indicates that these loci might be important genes for endophytism in *Burkholderia* spp., however, *in planta* experimentation is urgently necessary to confirm their possible involvement in plant colonization.

3.4 Concluding remarks

The genome of *B. kururiensis* M130 revealed a metabolically versatile bacterium able to prosper in the plant environment. Nitrogen fixation-related genes, production of ACC deaminase and some potential pathways for IAA synthesis were found in its genome possibly being in part responsible for its plant-growth promotion capability. Although no genes involved in the degradation of plant cell walls were unambiguously identified, this bacterium presents a large variety of attachment mechanisms and protein secretion systems that may contribute to its ability to invade and colonize the endosphere after penetration through natural root cracks. This genome also presents almost all of the genes suggested as important for endophytism in other *Burkholderia* spp. and it may contribute to other comparative genome analysis with the intent of better understanding the genetic requirements of an endophyte.

Table 3.2. Genes putatively responsible for endophytic behaviour in *Burkholderia* sp. (Ali et al., 2014) and their respective orthologous in *B. kururiensis* M130

Function	Gene ID *B. phytofirmans* PsJN	Position *B. kururiensis* M130 genome		
		contig	start	stop
Transporters				
Lysine exporter protein LysE/YggA	Bphyt_0034	5	1067406	1066819
Branched-chain amino acid ABC transporter ATPase	Bphyt_3906	1	252128	251361
Branched-chain amino acid ABC transporter inner membrane protein	Bphyt_3908	1	254972	253917
NAD(P)(+) transhydrogenase	Bphyt_4261	7	965162	963690
ABC transporter-like protein	Bphyt_4584	1	1044132	1045313
Major facilitator superfamily metabolite/H+ symporter	Bphyt_5520	1	1711146	1709851
Extracellular solute-binding protein family 1	Bphyt_5521	1	1709780	1708998
Gluconate 2-dehydrogenase (acceptor)	Bphyt_4638	9	610999	612390
Gluconate 2-dehydrogenase (acceptor)	Bphyt_4639	9	609213	610988
Gluconate 2-dehydrogenase (acceptor)	Bphyt_4640	9	608490	609209
Secretion and delivery system				
Type VI secretion protein	Bphyt_4913	8	184487	185026
Type VI secretion protein EvpB family	Bphyt_4914	8	185057	186550
Type VI secretion ATPase, ClpV1 family	Bphyt_4919	8	190688	193369
RND family efflux transporter MFP subunit	Bphyt_6992	1	1503214	1502018
Plant polymer degradation/modification				
Alpha/beta fold family hydrolase	Bphyt_6134	5	933180	932359
Alpha,alpha-trehalase	Bphyt_5350	5	1383141	1384883
Cupin	Bphyt_2288	4	170220	169708
Peptidase M48 Ste24p	Bphyt_3335		Not present	
Transcriptional regulators				
AsnC family transcriptional regulator	Bphyt_0434	1	558569	558036
Regulator protein FrmR	Bphyt_0109		Not present	
AraC family transcriptional regulator	Bphyt_2287	4	171059	170244
Two component winged helix family transcriptional regulator	Bphyt_4604	2	118281	119024
DeoR family transcriptional regulator	Bphyt_4951	2	121252	120470
LysR family transcriptional regulator	Bphyt_5523	1	1706657	1707574
LrgB family protein	Bphyt_5345	1	76633	75899
TrpR binding protein WrbA	Bphyt_6351	9	97929	97327
Detoxification				
Glutathione S-transferase	Bphyt_1366	4	525369	524668
Short-chain dehydrogenase/reductase SDR	Bphyt_1098	7	226717	227472
S-(hydroxymethyl)glutathione dehydrogenase/class III alcohol dehydrogenase	Bphyt_5114	1	905972	907078
2-dehydropantoate 2-reductase	Bphyt_5159		Not present	

Function	Gene ID *B. phytofirmans* PsJN	Position *B. kuruiensis* M130 genome		
		contig	start	stop
Redox potential maintenance				
Acetaldehyde dehydrogenase	Bphyt_1467	7	188428	189393
Carbonate dehydratase	Bphyt_2146	4	496486	495851
Aldehyde dehydrogenase	Bphyt_4023	5	856894	858345
Malate dehydrogenase	Bphyt_5456	5	1376354	1377472
3-hydroxyisobutyrate dehydrogenase	Bphyt_5931	9	648003	647113
Others				
hypothetical protein	Bphyt_5655	5	803189	803635
hypothetical protein	Bphyt_0435	1	558639	559580
2-isopropylmalate synthase	Bphyt_0573	1	676569	674872
Diaminopimelate decarboxylase	Bphyt_7089	8	48620	47358

4 Plant-induced gene expression in the rice endophyte *Burkholderia kururiensis* M130

4.1 Introduction

Rice (*Oryza sativa*) is the most important cereal feeding a large proportion of the world population (Ladha et al., 1997). However its production is not increasing at a rate that is adequate to satisfy the demands of the growing population. It is estimated that by 2020 it will be necessary to double the amount of nitrogen (N) fertilizer currently being used to achieve these higher yields (Ladha et al., 1997). An attractive alternative to chemical fertilizers in agriculture is the use of biofertilizers consisting of bacteria which can provide N to the plant via biological atmospheric N_2 fixation (BNF) (Ladha and Reddy, 1995; Reinhold-Hurek and Hurek, 1998).

The use of bioinoculants is currently growing at over 10% yearly in order to decrease the use of chemical additives in agriculture. Beneficial endophytes residing inside plants are attractive candidates for the development of bioinoculants as they increase plant growth and resistance to pathogens (Hardoim et al., 2008; Compant et al., 2010; Reinhold-Hurek and Hurek, 2011). It is believed that bacteria colonizing the plant interior might interact more closely with the host when compared to rhizospheric bacteria having less competition for nutrients and living in a more protected environment (Reinhold-Hurek and Hurek, 1998). Besides providing essential nutrients to plants, endophytes are also known to directly promote plant growth by the production and/or regulation of phytohormones (Hardoim et al., 2008; Reinhold-Hurek and Hurek, 2011).

The most predominant and studied endophytes belong to the three major phyla, Actinobacteria, Proteobacteria and Firmicutes and in addition to their *in planta* habitat they are also normally found in the soil/rhizosphere, which represents the main source of endophytic colonizers. These soil/rhizosphere inhabiting endophytes are thought to reach the inside of plants through the phyllosphere or through seeds (Compant et al., 2010). Endophytes can colonize different compartments of the plant apoplast, including the intercellular spaces of the cell walls and xylem vessels, and some of them are even able to colonize plant reproductive organs, such as seeds, which allows them to be vertically transmitted from one generation to the other (Compant et al., 2010). However, we currently have little knowledge on how bacteria adapt, colonize and live as endophytes in plants. Endophytes need to initially enter the plant endosphere, quickly adapt to the new environment and overcome plant defence responses (Hardoim et al.,

2008; Compant et al., 2010; Reinhold-Hurek and Hurek, 2011). Endophytic colonization is believed to depend in part on motility, root penetration and adaptation to the plant intercellular environment.

Endophytic *Burkholderia kururiensis* M130 was isolated from rice roots in Brazil and it was shown to promote plant growth at least in part due to the increase in nitrogen availability (Baldani et al., 1997a; Baldani et al., 1997b; Baldani et al., 2000). Inoculation experiments of rice with strain M130 have shown that up to 30% of the total nitrogen accumulated by the plant had been fixed by the bacteria, increasing rice growth and yield (Baldani et al., 1997a; Baldani et al., 2000), which makes it a potential candidate for use as a biofertilizer/bioinoculant. Recently, we have sequenced the genome of *B. kururiensis* M130 and observed that it possesses several genes potentially related to plant growth promotion, including the *accD* gene encoding 1-aminocyclopropane-1-carboxylate deaminase and the *nif* gene cluster (Coutinho et al., 2013a). However, as is currently the case for most identified endophytes, no information is available on the molecular mechanisms that play an important role in plant colonization by these bacteria.

Shidore and coworkers recently used whole genome microarray to investigate the response of the endophyte *Azoarcus* sp. strain BH72 to rice root exudate (Shidore et al., 2012). These experiments revealed that 4.4% of the total protein coding genes of the strain were differentially regulated in the presence of the exudate, including genes for pilin, signal transduction and type IV secretion system (Shidore et al., 2012). This work suggests that plant exudates may be an important switch for the endophytic lifestyle of these bacteria. Although this and a few other studies were able to identify genes differentially regulated in the rhizosphere or in the presence of plant root exudates (Rediers et al., 2003; Mark et al., 2005; dos Santos et al., 2010; Shidore et al., 2012), our knowledge on bacterial gene regulation occurring inside the plant is very limited. Cordeiro and coworkers performed proteomics experiments with endophytic *H. seropedicae* cultivated in the presence or absence of sugar cane extract (total plant macerate) and revealed proteins that might be differentially expressed inside the plant (Cordeiro et al., 2013). These proteins were mainly related to metabolic changes and adaptations, which are probably involved in the establishment of the endophytic

lifestyle. However, a general mode of regulation and target genes common to bacterial endophytes has not yet been identified.

In the present study we analysed the changes in gene expression of *B. kururiensis* M130 occurring during its interaction with the rice plant extract in order to have a better understanding of the mechanisms involved in endophytism in this model. This was achieved via a more traditional screening of a transposon promoter-probe mutant genome bank and by a genome-wide scale RNAseq approach.

4.2 Materials and Methods

4.2.1 Bacterial strains, plasmids, media and recombinant DNA techniques

B. kururiensis M130 wild type (Baldani et al., 1997a) rifampicin (Rif) resistant and its derivative strains were grown at 30°C in King's medium (KB) (King et al., 1954) or M9 minimal medium supplemented with glucose (Sambrook et al., 1989). Medium containing macerated rice material was prepared as follows: 20–25 g of rice plant were macerated in the presence of liquid nitrogen, added to 400 ml of KB or M9 medium, autoclaved and filtered to remove large plant materials. *E. coli* strains were grown in Luria-Bertani (LB) medium at 37°C. The plasmid pGEM-T Easy Vector (Promega, Madison, WI, USA) was used for cloning. Antibiotics were added when required at the following final concentrations: ampicillin (Amp), 100 μg ml^{-1}; kanamycin (Km), 50 μg ml^{-1} (*E. coli*) or 100 μg ml^{-1} (*B. kururiensis),* nitrofurantoin (Nf), 100 μg ml^{-1} and Rif, 100 μg ml^{-1}. The β-glucuronidase substrate X-GlcU (Life Technologies, Carlsbad, CA, USA) was incorporated in solid medium at 40 μg ml^{-1}.

All recombinant DNA techniques were performed as described previously (Sambrook et al., 1989). Plasmids were purified by using EuroGold columns (EuroClone, Italy); total DNA from *Burkholderia* was isolated by sarkosyl-pronase lysis as described previously (Better et al., 1983). Generated plasmids were sequenced-verified by Macrogen (Europe). The sequences of the primers used are given in Appendix Table 7.9.

4.2.2 Construction of the transposon promoter-probe genomic mutant bank in *B. kururiensis* and mapping of transposon insertion sites

Plasmid pCRS530 (Reeve et al., 1999) carrying the mTn5-GNm (containing *gusA*-P_{tac}-*nptII*-T_{trpA}) was harbored in *E. coli* S17-1 (Simon et al., 1983) and biparental conjugation was performed in order to conjugate this plasmid into *B. kururiensis* M130. The transconjugants harboring the mTn5-GNm into the chromosome were selected on KB plates containing Km, Nf and Rif and incubated 18 h at 30°C. Several conjugations were performed in order to obtain over 100,000 independent transposon insertions. The DNA sequence flanking transposon mutants in *B. kururiensis* M130 was determined

using an arbitrary PCR procedure as previously described (O'Toole and Kolter, 1998a). In this method, the DNA flanking the transposon insertion site was enriched in two rounds of amplification using primers (Appendix Table 7.9) specific to the ends of the mTn5-GNm element and primers of random sequence that annealed to chromosomal sequences flanking the transposon.

4.2.3 Total RNA isolation

RNA isolations were carried out from three independent cultures of *B. kururiensis* M130 grown in KB medium and KB medium supplemented with rice plant macerate. The cultures were incubated at 30°C, 180 rpm until they reached an OD_{600} of 3.0; this stage was chosen as it is the beginning of stationary phase. RNA isolation was carried out from 2 x 10^9 cells using the Ribopure™ bacteria RNA isolation kit (Ambion Inc., Austin, TX, USA) following the manufacturer's instructions. Isolated RNA was treated with DNase at 37°C for 1 h and purified. The purity of RNA was assessed by PCR on total RNA (250 ng) with GoTaq polymerase (Promega) using 16S_M130 primers (Appendix Table 7.9). RNA quality and concentration were assessed by Nanodrop (Thermo Scientific, Wilmington, DE, USA).

4.2.4 RNAseq experiment and analysis

RNAseq experiments were performed by IGA Technology Services Srl (Udine, Italy). Briefly, RNA samples were first treated with Ribo-Zero Magnetic Kit specific for Gram-negative bacteria (Epicentre, Madison, WI, USA) in order to deplete ribosomal RNA. 1.5 µg of total RNA was used as starting material following the standard protocol. The purification of rRNA-depleted samples was performed by using Agencourt RNAClean XP kit (Beckman Coulter, USA) as described in the manufacturer's protocol. rRNA-depleted RNA samples were then processed using Encore Complete Prokaryotic RNA-Seq Library Systems from Nugen (NuGEN Technologies, Inc., CA, USA). Pools of 6 samples were loaded on one lane of Illumina flowcell and clusters created by Illumina cBot. The clusters were sequenced on the Illumina HiSeq2000 (Illumina Inc.). One lane in 6-plex was run obtaining about 20 millions of single-reads per sample; each read was 50 bp long.

CLC-Bio Genomics Workbench software (CLC Bio, Denmark) was used to calculate gene expression levels based on Mortazavi *et al.* approach (Mortazavi et al., 2008) and fold changes between samples. Gene functions were annotated using the RAST-Server (Aziz et al., 2008). The cutoff FDR (false discovery rate)-adjusted *P* value used was 0.01 with a minimum 2-fold change.

4.2.5 Plant endophytic colonization assays

Rice seeds cv. Baldo were surface sterilized through 1 h wash with 2.5% sodium hypochlorite, followed by several washes with sterile water. Seeds were then aseptically transferred to sterile boxes with a thin layer of sterile water for seed pre-germination. Following incubation for five days at 28°C, in absence of light, the pre-germinated rice seeds were aseptically transferred to 50 ml Falcon tubes containing 35 ml of a modified Jensen's N-free plant nutrient medium (Somasegaran and Hoben, 1994) with 0.3% agar.

Plant infection and endophytic bacteria isolation was conducted as previously described (Mattos et al., 2008). Briefly, plantlets infection assays were carried out by inoculation of $2x10^7$ CFU into each Falcon tube containing rice plantlets. After incubation for 14 days with a 12-h photoperiod at 28°C, plantlets were collected and cut, in order to gather only the roots. The excised plant segments were subjected to surface sterilization with 1% sodium hypochlorite for 5 min, followed by several washes with sterile water. The roots were then weighed, transferred to sterile mortars containing 1 ml of sterile PBS and macerated with a pestle. From each suspension, a series of 10-fold dilutions were prepared using sterile PBS, and aliquots of 100 µl were spread plated onto KB medium containing Rif for the isolation of *B. kururiensis* M130 WT, or Rif and Km for the isolation of *B. kururiensis* M130 derivatives. Plates were incubated for 2 days at 28°C. Bacterial quantification was expressed as CFU/g of fresh weight plant tissue and eight replicates, from two independent colonizing assays were used to determine the average CFU values.

4.2.6 β-glucuronidase assays and histochemical staining for β-glucuronidase activity

β-Glucuronidase activities were determined as described previously (Ferluga and Venturi, 2009) with *B. kururiensis* mutants grown in the presence or absence of rice plant extract. All experiments were performed in triplicate, and the mean value is given.

For histochemical staining, at least three seedlings from two independent inoculations were collected 12 days after inoculation and their roots stained for β-glucuronidase activity in 50 mM potassium phosphate buffer (pH 7.0) containing 500 μg of X-GlcU ml^{-1} overnight at 37°C in darkness. The reaction was subsequently cleared in 70% ethanol, before visualization. β-glucuronidase expression was examined with an stereomicroscope (MZ125; Leica) and images recorded with a digital microscope camera (DFC420c; Leica).

4.3 Results

4.3.1 *B. kururiensis* M130 shows differential expression of chromosomal transcriptional gene fusions in response to rice plant extract

In order to determine the differentially expressed genes of *B. kururiensis* M130 in the presence of rice plant extract we constructed a promoter-probe genomic mutant bank of this strain using mTn5-GNm. This mTn5-based transposon possesses a kanamycin resistance gene and a promoterless *gusA* reporter gene. The latter allows to monitor gene expression when the transposon is inserted in the correct transcriptional orientation of an ORF (Reeve et al., 1999).

A total of 10,100 independent mutants from the saturated *B. kururiensis* M130 genomic mutant bank, constructed as described in the Materials and Methods section, were screened by patching them independently in duplicate in solid medium (containing X-GlcU) with or without macerated rice plant. Potential differentially regulated transposon insertions were selected on media plates by identifying colonies that displayed different levels of β-glucuronidase activity as observed by naked eye in a medium-dependent color display. Independent colonies, each representing a single transposon insertion fusion, were scored based on a white-blue scale indicating up-regulated or down-regulated loci in the presence of rice plant extract by the variation in the color intensity (Figure 4.1). This screen identified 61 transposon insertions showing differential regulation of which 36 were up-regulated and 25 were down-regulated in the presence of rice plant extract (Table 4.1).

In order to validate these observations, the 61 transposon insertion fusions identified by the screen on solid medium were then subjected to β-glucuronidase activity quantification after growth in shaking liquid media either in the presence or absence of macerated rice plant. In this experiment, 58 fusions showed differential expression in the presence of rice plant extract, with 36 being up-regulated and 22 down-regulated. The fold changes of enzyme activity varied from 1.34 to 20.91 (Table 4.1 and Appendix Table 7.10). Almost all the insertions displayed a similar expression pattern to what observed in solid media, interestingly, however, in the liquid enzyme assay three of the transposon insertions presented a different type of regulation. More

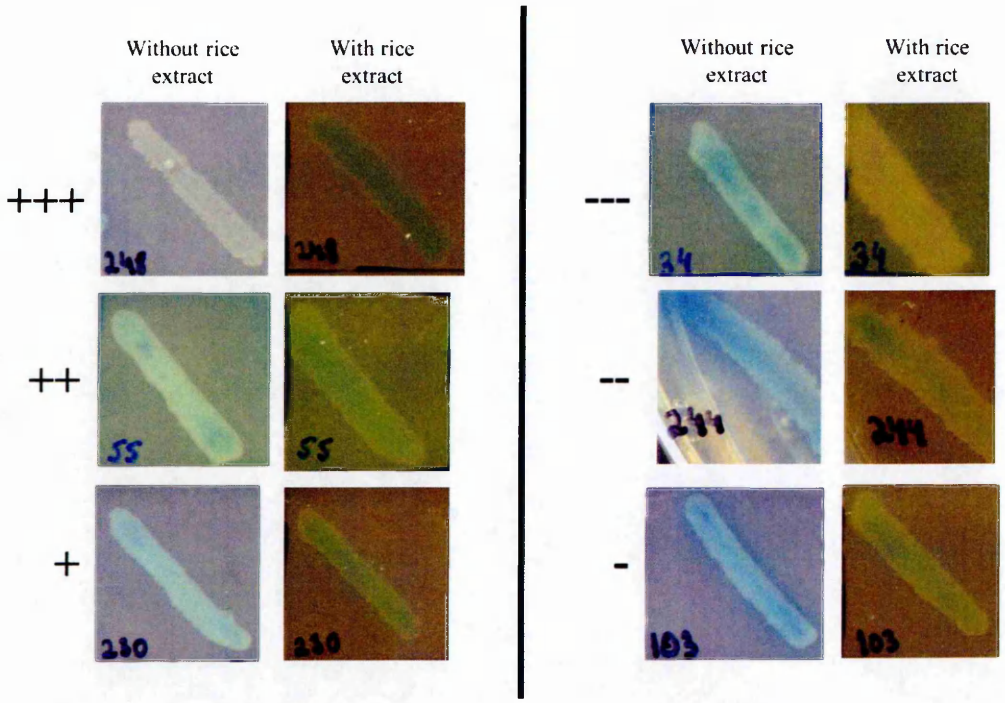

Figure 4.1. Scoring system created to categorize the *B. kururiensis* mTn5- GusNm fusions based on the type of regulation presented (up-regulated or down-regulated in the presence of rice plant extract) and the variation in color intensity. + or -, slightly up or down-regulated in the presence of rice plant extract, respectively; ++ or --, up or down-regulated in the presence of rice plant extract, respectively; +++ or --- strongly up or down-regulated in the presence of rice plant extract, respectively;

precisely, in the presence of rice plant extract, insertions 2 and 34 were down-regulated in solid medium and up-regulated in the liquid medium. In contrast to this observation, genomic insertion 160 displayed the opposite trend in solid and liquid conditions (Table 4.1). Moreover, three of the transposon insertions isolated on the solid media screen (*i.e.* mutants 19, 67 and 249) did not show differential regulation in the liquid enzyme assay (Table 4.1). These results suggest that the growth conditions (*i.e.* solid or liquid) might be important for regulation of these loci.

In order to identify the genes in the differentially expressed transposon insertion mutants selected above, the location of the transposon was mapped using an arbitrary PCR method (O'Toole and Kolter, 1998a). Using this procedure, we were able to map 49 out of the 61 mutants (Table 4.1). In order to map the other 12 transposon insertion

Table 4.1. *B. kururiensis* M130 mTn5-GusNm insertions that show differential expression of β-glucuronidase in the presence of rice plant extract.

Mutant #	Scaffold_site of transposon insertion[1]	ORF start-end	Predicted function	Plate experiment score[2]	Fold change (β-glucuronidase activity)[3]	Fold change (RNAseq)[3]	FDR value (RNAseq)
2	5_1145152 (-)	1145980-1144556	Permeases of the major facilitator superfamily	--	1.8	2.35	0.003
19	1_1316554 (-)	1316527-1315403	Porin	++	NS	-1.53	0.03
21	ND	ND	ND	+++	2.0	ND	ND
28[a]	1_984753 (-)	ND	Unknown	---	-1.98	ND	ND
30[a]	8_351373 (-)	ND	Unknown	++	2.16	ND	ND
31	5_301794 (-)	302442-301495	5-dehydro-4-deoxyglucarate dehydratase	++	4.86	5.16	2.7e-07
34[a]	7_928794 (-)	ND	Unknown	---	1.75	ND	ND
36[a]	7_351364 (-)	ND	Unknown	+	1.82	ND	ND
37	ND	ND	ND	+++	1.71	ND	ND
38	5_562245 (+)	561131-562321	P-hydroxybenzoate hydroxylase	+++	2.79	7.39	5.28e-18
43	5_1525790 (+)	1525761-1525934	Hypothetical protein	-	-1.93	LNR	LNR
47	4_312320 (-)	312375-311863	Hypothetical protein	-	-2.01	1.47	0.37
48	7_2367 (-)	2629-2150	Hypothetical protein	+	1.72	9.48	0.0005
50	8_154092 (+)	154059-155186	Hypothetical protein	-	-2.2	-2.12	3.8e-07
55	8_220645 (+)	220493-221701	Starvation sensing protein RspA	++	2.48	1.22	0.82
57	7_461873 (-)	462221-461949	Hypothetical protein	--	-2.4	3.0	0.0001
62	8_310198 (-)	310785-310081	Protocatechuate 3,4-dioxygenase beta chain	++	2.78	1.88	1.5e-11
64	ND	ND	ND	-	-1.94	ND	ND
65	5_301785 (-)	302442-301495	5-dehydro-4-deoxyglucarate dehydratase	++	6.02	5.16	2.7e-07
66[a]	7_941975 (+)	ND	Unknown	++	2.25	ND	ND
67	1_77081 (-)	77084-76680	Holin-like protein CidA	--	NS	1.3	0.8
71	5_1194214 (-)	1194214-1192490	L-lactate permease	---	-1.58	-1.24	0.62
73	8_319309 (-)	319413-318259	Porin	++	3.2	6.75	2.16e-61
76	1_1682424 (+)	1681913-1682509	Hypothetical protein	+	3.0	3.86	3.7e-09
79	5_1155999 (-)	1156180-1154984	Outer membrane porin protein 32 precursor, putative 3-hydroxyphenylpropionic acid porin	+	1.48	7.08	7.03e-11
87	1_980199 (+)	979982-980416	Hypothetical protein	--	-1.49	LNR	LNR

Mutant #	Scaffold_site of transposon insertion[1]	ORF start-end	Predicted function	Plate experiment score[2]	Fold change (β-glucuronidase activity)[3]	Fold change (RNAseq)[3]	FDR value (RNAseq)
99	1_1536526 (+)	1535599-1537965	Non-ribosomal peptide synthetase modules, pyoverdine-like	-	-9.66	-1.4	0.64
103	1_1024300 (+)	1023985-1026093	TonB-dependent receptor	-	-2.06	-31.4	1.37e-95
121	ND	ND	ND	+++	1.71	ND	ND
123	4_65298 (-)	66227-65079	Porin	++	3.7	2.78	7.27e-09
124	1_1528111 (+)	1516822-1533051	Non-ribosomal peptide synthetase modules, pyoverdine-like	-	-20.91	1.38	0.7
130	ND	ND	ND	+++	1.9	ND	ND
131	5_1086991 (+)	1086275-1087477	Acetyl-CoA acetyltransferase Beta-ketoadipyl CoA thiolase	-	-2.25	-1.33	0.12
135	ND	ND	ND	+++	2.03	ND	ND
137	5_856719 (+)	856003-856830	4-hydroxycinnamoyl CoA hydratase/lyase	+	6.21	2.99	8.8E-07
144	5_1487519 (-)	1487503-1487195	Response regulator receiver protein	--	-4.04	9.82	0.0
145	9_92313 (-)	92762-91146	RND efflux system, outer membrane lipoprotein, NodT family	+	1.92	1.42	0.123
146	5_301785 (-)	302442-301495	5-dehydro-4-deoxyglucarate dehydratase	++	6.51	5.16	2.7E-07
150	9_88340 (-)	88688-87810	Membrane fusion component of tripartite multidrug resistance system	-	-2.2	-2.2	2.01E-07
160	ND	ND	ND	++	-1.55	ND	ND
163	1_1005739 (+)	1005244-1005789	XdhC protein (assists in molybdopterin insertion into xanthine dehydrogenase)	+	2.5	9.76	0.00995
164	5_303947 (+)	303844-304677	UDP-glucose 4-epimerase	++	3.79	8.62	9.34e-07
178	7_512666 (+)	512658-512861	Hypothetical protein	-	-2.62	41.21	3.2e-12
187	1_715354 (+)	714919-715893	Threonine dehydratase, catabolic	-	-2.05	-2.23	4.48e-08
193	8_220645 (+)	220493-221701	Starvation sensing protein RspA	+++	2.97	1.22	0.82
194	ND	ND	ND	+++	1.95	ND	ND
201	5_652044 (-)	652547-651849	Phosphatase CheZ	-	-1.7	4.38	5.82e-13
219	ND	ND	ND	+++	1.93	ND	ND
226	1_1543336 (+)	1541329-1543323	Ferric hydroxamate ABC transporter, permease component FhuB or 4'-phosphopantetheinyl transferase	--	-17.6	-1.65	0.45
227[a]	5_90359 (+)	ND	Unknown	++	1.82	ND	ND
230	1_911723 (+)	911073-911855	Sorbitol dehydrogenase	+	2.0	4.6	1.59e-20
243	7_306591 (+)	306335-309208	Extracellular Matrix protein PelA	-	-2.12	-1.03	0.86

Mutant #	Scaffold_site of transposon insertion[1]	ORF start-end	Predicted function	Plate experiment score[2]	Fold change (β-glucuronidase activity)[3]	Fold change (RNAseq)[3]	FDR value (RNAseq)
244	1_793114 (-)	793611-792112	Glycerol kinase	--	-2.02	-2.42	7.48e-11
248	ND	ND	ND	+++	1.34	ND	ND
249	4_96716 (-)	97427-96465	Agmatinase	--	NS	-1.43	0.45
250	1_625434 (+)	624594-626030	Ammonium transporter	-	-2.32	4.04	5.06e-13
251	ND	ND	ND	+++	1.37	ND	ND
262	ND	ND	ND	+++	1.47	ND	ND
267	5_301748 (-)	302442-301495	5-dehydro-4-deoxyglucarate dehydratase	++	5.7	5.16	2.7e-07
270	1_1165866 (+)	1164892-1166553	3-methylmercaptopropionyl-CoA ligase (DmdB)	+	2.2	2.13	2.45e-07
275[a]	5_90553 (+)	ND	Unknown	++	1.76	ND	ND

[1] (+) or (-) indicates the DNA strand of the *B. kururiensis* M130 genome in which the coding strand of the *gusA* gene of the transposon insertion was inserted; [2] Scale based on Figure 1; [3] Negative and positive values represent down- and up-regulation in the presence of rice plant extract respectively; [a] Mutants that have the transposon inserted opposite to the transcriptional orientation of the annotated gene (antisense transcripts); NS, not statistically different; ND, not determined; LNR, low number of reads.

fusions we also performed genomic digestions and subcloning aiming to isolate the genomic region flanking the Km resistance gene of the inserted transposon. However, this approach was also not successful for these 12 insertions; the reason for this is currently unknown. It is possible that the DNA regions associated with the location of the transposon contain gene(s) that have deleterious effects when over-expressed in *E. coli*.

The results obtained from the 49 mapped transposon insertions revealed that most of the genes that were differentially expressed in the presence of rice macerate were related to cellular processes and metabolism, including genes involved in the degradation of aromatic compounds which are known to be abundant in the plant (*e.g.* p-hydroxybenzoate hydroxylase and protocatechuate 3,4-dioxygenase beta chain) (Figure 4.2). The second most abundant group were genes involved in transport of molecules across membranes, which are believed to be important for the exchange of nutrients within the plant (Figure 4.2). Interestingly, in seven transposon insertion mutants the *gusA* gene mapped in the opposite orientation with respect to transcriptional direction of an annotated gene of the *B. kururiensis* M130 genome (Table 4.1 and 4.2, Figure 4.2). The presence of antisense transcripts could be related to the existence of unidentified reading frames in the opposite strand of currently annotated gene, however none of the potential ORFs identified encode peptides displaying similarity to any other known protein, which could indicate the presence of a non-coding RNA (ncRNA) in these regions (Table 4.1). Two of these transposon insertions map at different locations on the opposite orientation of the same gene (*i.e.* transposon insertions 227 and 275) encoding a putative transmembrane protein (Table 4.2). Several other transposon insertions also mapped to the same locus (Table 4.1) as is the case for four different insertions in the gene encoding a 5-dehydro-4-deoxyglucarate dehydratase (*i.e.* insertions 31, 65, 146 and 267, with insertions 31 and 146 being siblings; Table 4.1). This gene is most likely involved in the degradation of D-galacturonate, which is one of the major sugars present in plant cell walls. Interestingly, all the genes identified that were likely to be involved in iron uptake were down-regulated in the presence of rice plant extract. A transposon (insertion 144) was also found in a gene coding for a response regulator receiver protein; this gene could be possibly involved in transcriptional regulation in the presence of plant macerate (Table 4.1). The gene

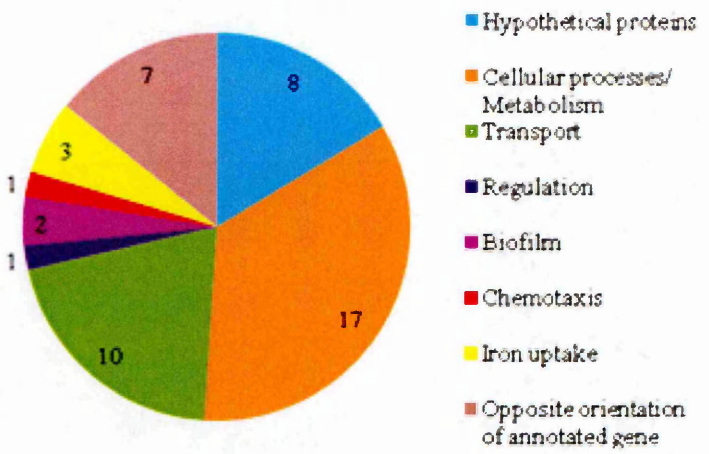

Figure 4.2. Functional classification of the genes showing differential expression of β-glucuronidase in the presence of rice plant extract.

encoding this protein seems to be part of an operon with a transcriptional regulator of the Crp/Fnr family (Figure 4.3). In summary, this genetic screen revealed that, in the presence of rice macerate, *B. kururiensis* M130 differentially regulates several metabolism-related genes and membrane transporter genes that are likely to allow this endophyte to cope with the new environmental conditions.

4.3.2 *In planta* studies of the promoter probe transposon insertions

It was of interest to perform *in planta* studies with some of the transposon insertion mutants in order to evaluate the role of these genes in endophytic colonization and their expression in the plant environment. In order to carry out these experiments in rice plants the mutants 146 and 193, which had transposon insertions in loci that were highly up-regulated in the previous screen, were used (Table 4.1). Results showed that the mutants were able to endophytically colonize the roots of rice plants at the same level as the wild-type strain (Figure 4.4A). The roots of plants inoculated with mutant 146 showed strong blue coloration when stained for β-glucuronidase activity with the lateral roots displaying a more intense coloration (Figure 4.4B). This qualitative result confirms high expression of the gene encoding a 5-dehydro-4-deoxyglucarate

Table 4.2. ***B. kururiensis*** **M130 mTn5-GusNm insertions that had the transposon inserted in the opposite orientation of the annotated gene.**

Mutant #	Scaffold_site of transposon insertion	Annotated ORF		Strand transposon/ annotated ORF	Predicted function of the annotated ORF	Fold change (RNAseq)[1]	FDR value (RNAseq)
		Start	End				
28	1_984753	984226	984864	-/+	Alkyl hydroperoxide reductase subunit C-like protein	1.34	4.1e-08
30	8_351373	350600	351676	-/+	Dipeptide transport system permease protein DppB	2.89	0.00013
34	7_928794	928620	928934	-/+	Hypothetical protein	2.83	1.9e-07
36	7_351364	351147	352178	-/+	2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase	-1.47	0.16
66	7_941975	941976	940525	+/-	Catalase	1.21	0.48
227	5_90359	90922	90299	+/-	Probable transmembrane protein	1.04	0.9
275	5_90553	90922	90299	+/-	Probable transmembrane protein	1.04	0.9

[1] Gene expression values expressed as fold change; negative and positive values represent down- and up-regulation in the presence of rice plant extract, respectively.

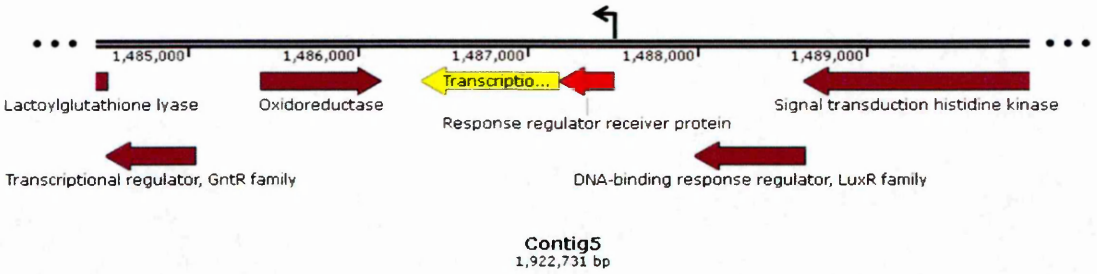

Figure 4.3. Graphical representation of the region of transposon insertion 144 in *B. kururiensis* M130 mTn5-GNm. Genes were annotated using the RAST-Server. The black arrow represents the insertion site of the transposon and the orientation of its *gusA* gene. The gene in yellow is a transcriptional regulator of the Crp/Fnr family.

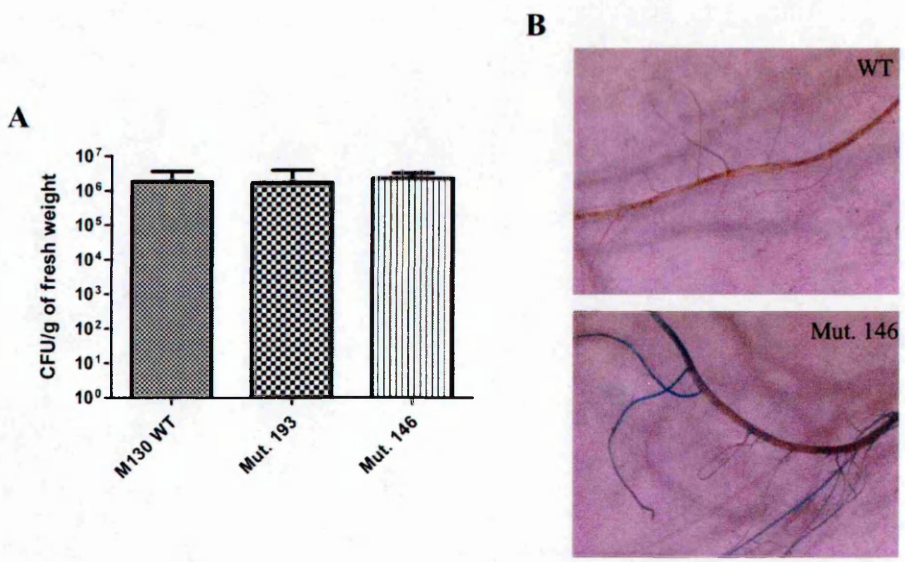

Figure 4.4. Rice colonization assays performed with *B. kururiensis* M130 (WT) or its mTn5-GusNm mutants 146 and 193. (A) Endophytic root colonization levels; bacterial endophytic colonization was measured by grinding and plating surface-sterilized roots after 14 days, and CFU/g levels of 8 replicates from 2 independent experiments are plotted. Bars indicate means ± standard deviations. (B) β-glucuronidase (GUS) staining of roots of rice plants 14 days after inoculation with wild-type strain or with mutant 146. Control plants inoculated with the WT strain showed no GUS activity. GUS activity on plants inoculated with mutant 146 was observed on all roots, with the most intense color development on the lateral roots.

dehydratase *in planta*. However, roots of plants inoculated with mutant 193 did not show blue coloration upon staining behaving as the WT strain, which indicates that the gene encoding the starvation sensing protein, RspA, is either not expressed *in planta* or more likely that the expression is too low to be detected by this staining methodology. In conclusion neither of these two loci played a crucial role for endophyte colonization; however one of them displayed high expression on the surface of roots pointing to a role for this gene *in planta*.

4.3.3 *B. kururiensis* M130 transcriptome in response to rice plant extract

In order to perform a comparative analysis of *B. kururiensis* M130 response to rice macerate extract by different methods and to obtain overall transcriptional profiling of this rice endophyte in plants, we performed strand-specific RNAseq analysis. Total RNA was purified from *B. kururiensis* grown in rich liquid media in the presence and absence of macerated rice extract as described in the Materials and Methods section.

4.3.3.1 Comparison of the RNAseq data with the transposon-insertion promoter-probe assay

The major reason for performing the RNAseq experiment was to validate the data obtained via the genetic screen of the transposon promoter-probe insertions in response to rice macerate. We therefore carefully compared the RNAseq data with the 49 mapped transposon insertions keeping in mind that the growth media used in the two experimental set-ups was identical. There are however two differences among the two experiments to consider, (i) the genetic screen was performed using colonies in solid media, whereas the RNAseq data resulted from bacterial RNA purified from bacteria grown in shaking liquid media; in order to make the comparison closer, the results generated with the RNAseq were compared to the ones derived from the β-glucuronidase enzyme quantification of the transposon insertions obtained when bacteria were also grown in liquid shaking cultures, and (ii) the screening using the transposon genomic library meant that each CFU was a knock-out mutant in a specific locus which could have a direct or indirect effect on its own expression. This data

comparison revealed that 27 out of the 49 loci that were differentially expressed in the genetic screen also showed a similar trend of differential gene expression profiles in the RNAseq experiment with an FDR value ≤ 0.01 (Table 4.1). These loci are mostly related to metabolism (especially of aromatic compounds) and different types of membrane transport systems. However, loci 57, 144, 178, 201 and 250 identified in the transposon screen presented a different type of regulation in the RNAseq experiment. The β-glucuronidase activity assays of these five transposon insertions showed a down-regulation of these genes in the presence of rice plant extract, whereas in the RNAseq data they were being up-regulated (Table 4.1). These results might suggest that the products of these genes may be involved in their own regulation, as mutations on them might affect their pattern of expression.

The three transposon insertion loci which displayed differential expression only on plate solid medium and not in β-glucuronidase liquid assays (*i.e.* mutants 19, 67 and 249), were also not differentially expressed in the RNAseq experiment which is in accordance as the RNAseq was also performed in shaking liquid media (Table 4.1). The expression of these three loci is therefore influenced by static growth in solid media and not liquid shaking growth.

The regions of the seven transposon insertions identified as antisense transcripts were also analysed on the RNAseq data and the presence of complementary RNA reads was confirmed for all but two (*i.e.* insertions 36 and 66; Appendix Figure 7.5). However in the absence of data corresponding to the size of the transcripts, no quantification could be performed. Interestingly, in the case of three antisense transcripts (*i.e.* insertions 28, 30 and 34), their sense annotated ORFs were also shown to be differentially regulated in the presence of rice plant macerate in the RNAseq results (Table 4.2).

We were not able to properly evaluate the expression of two loci of the transposon genetic screen as the number of RNA reads for these loci was below the quality threshold (*i.e.* for insertions 43 and 87) (Table 4.1).

Interestingly, the gene encoding the starvation sensing protein RspA identified in transposon insertions 55 and 193 and which was up-regulated in the presence of rice plant extract in the genetic screen, was not differentially expressed in the RNAseq experiment (Table 4.1). In the *in planta* studies described above we did not detect

expression using staining methodology of the root colonized by this strain carrying the transposon insertion. Thus it is likely that the mutation on this gene affects its own expression.

4.3.3.2 Global analysis of the RNAseq transcriptome studies

Results of the RNAseq experiment showed that the expression of a large number of genes was significantly altered (FDR value ≤ 0.01) in response to rice macerate, with 1,825 genes being differentially expressed by 2-fold or more (Appendix Table 7.11) representing 27.7% of the protein-coding genes of *B. kururiensis* M130. Moreover, 69% of the differentially expressed genes were up-regulated (Figure 4.5) and 31% down-regulated.

The highest percentage of differentially regulated genes corresponded to hypothetical proteins (24.7%), genes involved in various cellular processes and metabolism (22%) and genes involved in secretion and transport (13.8%) (Figure 4.5). Interestingly, 7.8% of the differentially expressed genes were transcriptional regulators of several different regulatory families (Figure 4.5), which gives a 1:12.8 ratio of genes coding for regulatory proteins to target genes. Moreover, 2.4% of the differentially regulated genes (predominantly up-regulated) were found to code for proteins involved in degradation or biosynthesis of plant-related compounds; these include genes involved in the degradation of aromatic compounds such as vanillic and salicylic acids and, genes involved in the production of molecules important for plant-growth promotion such as IAA and phenazines (Table 4.3). Genes potentially involved in plant adhesion and colonization (*e.g.* type IV pili) as well as genes coding for efflux pumps were also found to be differentially regulated in the presence of plant macerate. Some of the pumps belong to resistance nodulation and cell division family (RND) efflux systems that might be involved in bacterial defense against toxic-plant metabolites (Table 4.3). Other loci that might be involved in plant-bacteria interactions were related to chemotaxis, flagella, the type VI secretion system and the BraI/R quorum sensing system (Table 4.3).

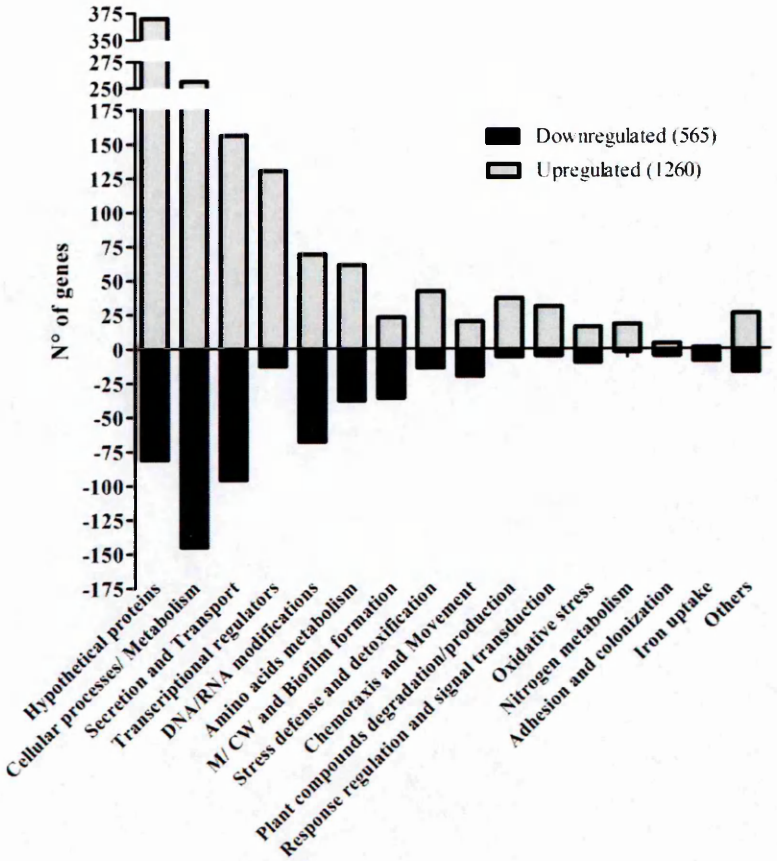

Figure 4.5 Functional classification of differentially expressed genes in *B. kururiensis* M130 in response to rice plant extract by transcriptome analysis. Only genes with a fold difference greater than 2 are included. M, membrane; CW, cell wall.

Table 4.3. Genes of *B. kururiensis* M130 coding for type VI secretion system, motility and chemotaxis, quorum sensing, plant compounds degradation/production, adhesion and colonization and efflux systems components that are differentially regulated in the presence of rice plant extract.

Gene location			Gene function	Fold change	FDR value
Contig	Start	Stop			
Type VI secretion system					
1	39644	42412	Putative Vgr-related protein	-6.94	3.01E-29
1	1728265	1730187	VgrG protein	-2.47	0.0016
5	1745301	1743430	Protein ImpG/VasA	8.06	0.0
5	1748897	1745298	type VI secretion system protein ImpL	11.17	0.0
5	1828225	1826873	Uncharacterized protein ImpJ/VasE	3.64	0.00003
5	1831019	1833355	VgrG protein	9.87	0.0
7	92308	94839	Putative Vgr-related protein	-4.15	3.18E-07
8	181365	180574	Type VI secretion system protein ImpK	-3.88	6.53E-12
8	182708	181362	Uncharacterized protein ImpJ/VasE	-3.45	1.28E-13
8	183414	182800	Type VI secretion lipoprotein/VasD	-3.78	2.86E-08
8	184487	185026	Uncharacterized protein ImpB	-3.69	7.22E-75
8	185057	186550	Uncharacterized protein ImpC	-4.74	1.93E-187
8	186626	187129	Uncharacterized protein ImpD	-9.32	1.24E-06
8	187200	187679	Uncharacterized protein ImpF	-5.44	2.05E-12
8	187740	189581	Protein ImpG/VasA	-4.83	2.74E-42
8	189545	190639	Uncharacterized protein ImpH/VasB	-9.31	5.76E-151
8	190688	193369	ClpB protein	-9.04	7.52E-228
8	193373	194524	Uncharacterized protein ImpA	-7.53	3.37E-180
8	200528	196605	Type VI secretion protein VasK	-2.86	2.26E-42
Motility and chemotaxis					
1	87713	89455	Flagellar M-ring protein FliF	-2.94	0.00014
1	89525	90526	Flagellar motor switch protein FliG	2.36	2.61E-13
1	90519	91199	Flagellar assembly protein FliH	2.47	0.0025
1	100577	100308	Flagellar biosynthesis protein FliQ	-7.16	0.00025
1	101416	100604	Flagellar biosynthesis protein FliP	-3.35	1.88E-17
1	103411	102413	Flagellar motor switch protein FliM	-8.7	7.84E-06
1	103990	103490	Flagellar biosynthesis protein FliL	-4.66	1.65E-23
1	109909	108977	Flagellar protein FlgJ	-8.58	4.48E-48
1	111121	109922	Flagellar P-ring protein FlgI	-8.37	3.4E-08
1	112650	111862	Flagellar basal-body rod protein FlgG	-7..68	1.75E-13
1	113449	112691	Flagellar basal-body rod protein FlgF	-10.93	5.13E-68
1	114972	113467	Flagellar hook protein FlgE	-9.53	9.98E-98
1	115777	115055	Flagellar basal-body rod modification protein FlgD	-5.31	0.000013
1	116223	115798	Flagellar basal-body rod protein FlgC	-6.4	1.17E-26
1	116936	116442	Flagellar basal-body rod protein FlgB	-4.17	0.0015
1	119021	119374	Negative regulator of flagellin synthesis	2.98	0.00085

Gene location			Gene function	Fold change	FDR value
Contig	Start	Stop			
1	122651	121872	Flagellar biosynthesis protein FlhG	-5.76	1.48E-06
1	124860	122761	Flagellar biosynthesis protein FlhF	-2.09	0.00083
1	127380	125281	Flagellar biosynthesis protein FlhA	-3.34	8.43E-08
1	128555	127377	Flagellar biosynthesis protein FlhB	-3.3	0.00056
1	142126	141092	Flagellar motor rotation protein MotB	2.88	2.62E-08
1	143009	142149	Flagellar motor rotation protein MotA	3.03	0.0039
1	144217	143915	Flagellar transcriptional activator FlhD	3.65	3.2E-10
1	405928	406470	Chemotaxis protein CheY	6.56	0.0011
4	271412	269751	Methyl-accepting chemotaxis protein	3.83	0.0
4	324426	326048	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	3.75	0.0
4	336512	334482	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	4.82	0.0
5	415368	416933	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	2.63	8.02E-06
5	440647	442200	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	2.98	0.0001
5	652547	651849	Chemotaxis response - phosphatase CheZ	4.38	5.82E-13
5	653949	655622	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	5.76	1.22E-06
5	655639	656181	Positive regulator of CheA protein activity (CheW)	3.79	0.0001
5	658164	657355	Chemotaxis protein methyltransferase CheR	11.8	4.0E-13
5	660244	658223	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	3.02	0.00057
5	660830	660297	Positive regulator of CheA protein activity (CheW)	3.39	1.66E-07
5	664808	663645	Methyl-accepting chemotaxis protein	5.34	3.88E-11
5	1128630	1128310	Flagellar transcriptional activator FlhD	3.6	0.0
5	1349858	1348815	Flagellar motor rotation protein MotA	4.57	2.78E-10
5	1875368	1873485	Chemotaxis protein CheY	-2.98	3.03E-21
7	198982	197438	Methyl-accepting chemotaxis sensory transducer	3.26	8.27E-06
8	363068	361518	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	3.5	9.09E-08
9	13641	15776	Methyl-accepting chemotaxis protein	-2.71	0.0099
9	15773	16429	Chemotaxis motB protein	-2.79	7.57E-10
BraI/R quorum sensing system					
5	543668	543078	Autoinducer synthesis protein LuxI	2.2	2.42E-06
5	544860	544132	Transcriptional activator protein LuxR	2.82	1.14E-07
Plant compounds degradation/production					
1	3489	3773	Phenylacetate-CoA oxygenase, PaaH subunit	2.75	7.33E-10

Gene location			Gene function	Fold change	FDR value
Contig	Start	Stop			
1	3782	4585	Phenylacetate-CoA oxygenase, PaaI subunit	2.39	0,0049
1	4632	5204	Phenylacetate-CoA oxygenase, PaaJ subunit	2.12	0,0078
1	5207	6295	Phenylacetate-CoA oxygenase/reductase, PaaK subunit	2.17	2.9E-06
1	82208	83626	Coniferyl aldehyde dehydrogenase	3.14	2.73E-14
1	893569	894204	Isochorismatase hydrolase	8.44	0.0036
1	989892	991220	Homogentisate 1,2-dioxygenase	8.79	3.69E-12
1	991217	992584	Fumarylacetoacetase	4.97	2.52E-06
1	1130463	1131614	3-dehydroquinate synthase	3.75	5.48E-06
1	1489183	1487903	Salicylate hydroxylase	-2.49	0.0035
1	1491920	1492849	2,3-dihydroxybiphenyl 1,2-dioxygenase	2.51	0.0044
1	1493794	1495251	Probable vanillin dehydrogenase oxidoreductase protein	3.32	9.4E-06
2	64972	63386	Cyclohexanone monooxygenase	7.64	2.48E-15
4	39188	37449	Salicylate hydroxylase	3.35	9.24E-20
4	392283	391372	Phenazine biosynthesis protein PhzF like	2.03	0.0007
5	61386	60853	2,4'-dihydroxyacetophenone dioxygenase	16.31	0.0022
5	336400	337281	2-polyprenylphenol hydroxylase and related flavodoxin oxidoreductases	7.87	5.59E-07
5	561131	562321	P-hydroxybenzoate hydroxylase	7.39	0
5	648731	647892	putative dioxygenase	13.36	1.52E-13
5	687208	688845	Salicylate hydroxylase	-2.37	7.23E-07
5	856003	856830	4-hydroxycinnamoyl CoA hydratase/lyase (Enoyl-CoA hydratase/lyase)	2.99	8.8E-07
5	856894	858345	Probable vanillin dehydrogenase oxidoreductase protein	2.47	2.7E-06
5	863363	862404	Vanillate O-demethylase oxidoreductase	24.09	0
5	864547	863501	Probable vanillate O-demethylase oxygenase subunit oxidoreductase protein	23.82	0
5	1026374	1027189	Fumarylacetoacetate hydrolase family protein	8.41	0.0001
5	1246946	1245492	Indoleacetamide hydrolase	3.29	1.9E-10
5	1637422	1638270	2-hydroxy-6-oxo-2,4-heptadienoate hydrolase	15.57	1.17E-14
5	1698016	1698501	2-polyprenylphenol hydroxylase and related flavodoxin oxidoreductases	3.31	0
7	142716	141775	Gentisate 1,2-dioxygenase	-2.22	0.0022
7	217887	220217	probable bifunctional hydroxylase/oxidoreductase	4.09	0.0028
7	754267	753284	2-keto-4-pentenoate hydratase	2.46	0.000027
7	796988	795825	Salicylate hydroxylase	2.59	2.52E-09
7	803826	805508	2,3-dihydroxybenzoate-AMP ligase	8.24	0.0005

Gene location			Gene function	Fold change	FDR value
Contig	Start	Stop			
7	806576	807331	3-oxoacyl-[acyl-carrier protein] reductase	5.36	1.94E-08
7	807347	808657	p-cumate dioxygenase large subunit (CmtAb)	5.58	1.17E-14
7	808668	809174	p-cumate dioxygenase small subunit (CmtAc)	3.73	8.12E-06
7	811256	812917	Salicylate hydroxylase	8.99	0
7	814198	815061	Beta-ketoadipate enol-lactone hydrolase	2.41	2.48E-15
8	453108	452671	Protocatechuate 4,5-dioxygenase beta chain	3.17	0.0023
8	525463	526626	4-carboxymuconolactone decarboxylase	6.58	0
9	41089	41586	putative 4-hydroxybenzoyl-CoA thioesterase	-3.26	0.0095
9	110415	111119	Succinyl-CoA:3-ketoacid-coenzyme A transferase subunit A	2.85	2.24E-10
9	111122	111763	Succinyl-CoA:3-ketoacid-coenzyme A transferase subunit B	2.41	0.0037
Plant adhesion and colonization					
7	101691	111191	Putative large exoprotein involved in heme utilization or adhesion of ShlA/HecA/FhaA family	-2.96	1.18E-26
1	460004	458961	Hemagglutinin-like transmembrane protein	3.79	2.62E-08
7	497545	497727	Flp pilus assembly protein, pilin Flp	2.97	7.51E-14
7	498366	498941	Flp pilus assembly protein TadG	-2.13	0.0002
7	499931	501361	Type II/IV secretion system secretin RcpA/CpaC, associated with Flp pilus assembly	-2.59	0.0001
7	502736	504163	Type II/IV secretion system ATP hydrolase TadA/VirB11/CpaF, TadA subfamily	-3.78	3.5E-06
3	204025	206622	Type II/IV secretion system secretin RcpA/CpaC, associated with Flp pilus assembly	2.39	0.0022
3	207052	208266	Type II/IV secretion system ATPase TadZ/CpaE, associated with Flp pilus assembly	6.83	0.0085
5	1898481	1892356	Alpha-2-Macroglobulin	-2.15	1.58E-16
Efflux system components					
1	896661	899873	RND efflux system, inner membrane transporter CmeB	-2.61	0.0001
1	1477589	1478998	RND efflux system, outer membrane lipoprotein CmeC	5.44	0.001
1	1561901	1560897	Predicted membrane fusion protein (MFP) component of efflux pump, membrane anchor protein YbhG	10.89	0.0
5	622727	624226	Outer membrane efflux protein precursor	-4.4	4.98E-27
5	884704	883211	RND efflux system, outer membrane lipoprotein CmeC	33.39	0.0

Gene location			Gene function	Fold change	FDR value
Contig	Start	Stop			
5	1124949	1126508	RND efflux system, outer membrane lipoprotein CmeC	3.56	0.000048
5	1445816	1447201	NodT family RND efflux system outer membrane lipoprotein	34.17	2.25E-10
5	1719455	1720954	RND efflux system, outer membrane lipoprotein, NodT family	-3.71	0.00013
7	45182	44160	Efflux transporter, RND family, MFP subunit, AcrA/E family	96.97	3.4E-06
7	292982	291834	Probable Co/Zn/Cd efflux system, MFP	2.14	6.41E-06
7	397250	394134	Cobalt-zinc-cadmium resistance protein CzcA; Cation efflux system protein CusA	-3.93	3.37E-26
7	398501	397308	Probable Co/Zn/Cd efflux system membrane fusion protein	-3.04	0.000059
7	477597	476038	Inner membrane component of tripartite multidrug resistance system	-2.08	1.13E-18
7	478908	477673	Membrane fusion component of tripartite multidrug resistance system	-2.13	1.33E-14
8	447600	446095	Outer membrane component of tripartite multidrug resistance system	4.82	4.17E-08
8	448382	449512	Probable Co/Zn/Cd efflux system membrane fusion protein	6.16	0.00011
8	449509	452607	Cation efflux system protein	3.27	0.000038
9	88688	87810	Membrane fusion component of tripartite multidrug resistance system	-2.2	2.01E-07
9	450758	453178	Drug efflux pump, RND superfamily	-6.46	4.92E-09

4.4 Discussion

In this study, we have used a transposon-promoter-reporter-system and RNAseq to study gene expression of the endophyte *B. kururiensis* M130 when exposed to rice plant macerate. To interpret this data one has to keep in mind that the transposon insertion might have an effect on the phenotype of the screen since it inactivates the gene (or possibly genes if there is an operonic organization) in which it inserts. This is not the case for the transcriptomic experiments using strand-specific RNAseq in which the wild-type strain was used. The RNAseq experiment provided a global view of the gene expression profile and also validated the genetic screen of the transposon promoter-probe library. Another remarkable difference of the two experimental set-ups is that in one the screen involves bacteria grown on solid-plate media, whereas the RNAseq experiment indicates gene expression when bacteria are grown in liquid-shaking cultures. Moreover, in addition to annotated genes, the combination of both techniques allowed us to also identify antisense transcripts potentially involved in plant-bacteria interactions.

Both experimental set-ups presented here used total autoclaved rice macerate as source of plant molecules. As *B. kururiensis* M130 is an endophyte which can live inside the rice plant in different locations and tissues, we believe that bacteria will be exposed to many different plant produced molecules, hence using complete plant macerate should provide a gene expression profile that includes all the important/major loci. It is likely however that some plant components to which *B. kururiensis* responds to might have been inactivated or degraded by autoclaving the macerate. Preparation of the rice macerate performed here has been described previously and it allowed the identification of a novel subfamily of LuxR regulators that responds to plant compounds (Ferluga et al., 2007; Ferluga and Venturi, 2009; Subramoni et al., 2011).

Results presented here indicate that the *B. kururiensis* endophyte undergoes major changes in its metabolism when exposed to plant macerate, probably as a result of the availability of a new range of nutrients and signals. This result is in agreement with observations from transcriptome and proteomic analysis of other bacteria growing in the presence of root exudates and plant extracts. In these reports, a major change in the expression of genes related to different aspects of metabolism, such as aromatic compound catabolism, energy generation and amino acid biosynthesis and metabolism

was observed (Mark et al., 2005; Shidore et al., 2012; Cordeiro et al., 2013). In addition, differential expression of several loci encoding for families of transporter proteins have also been observed indicating that several molecules are most probably transported inside or outside bacteria when they grow *in planta*.

Both experimental approaches identified that loci involved in the uptake of iron were down-regulated in the presence of rice macerated extract. The medium used for these experiments (*i.e.* King's B medium) is poor in available iron when compared to other rich media (King et al., 1954), thus these results indicate that the plant extract provides iron to *B. kururiensis* decreasing the expression of genes involved in iron uptake.

The gene encoding for a response regulator receiver protein identified with the transposon-reporter system (*i.e.* transposon insertion 144) was shown to be also highly up-regulated in the presence of plant macerate, according to the RNAseq experiment (Table 4.1). Interestingly the RNAseq data revealed that also the transcriptional regulator located downstream of this locus which belongs to the Crp/Fnr superfamily (*i.e.* 8.6 fold-change) was also up-regulated (Figure 4.3). The Crp/Fnr family of regulators are often involved in the regulation of bacterial metabolic versatility, virulence factors, degradation of aromatic compounds, nitrogen fixation, photosynthesis and various types of respiration and stress-related features. They are able to respond to a broad spectrum of intracellular and exogenous signals such as cAMP, anoxia, oxidative stress, carbon monoxide or temperature (Körner et al., 2003) and in plant-related bacteria this family of regulators is well known for its role in the regulation of the symbiotic nitrogen fixation process in rhizobia (Fischer, 1994).

Interestingly, the type VI secretion system (T6SS) of *B. kururiensis* M130 is down-regulated in the presence of plant rice extract. The T6SS in bacteria has recently been identified and its mode of action is still being characterized; it is known however that it can power secretion of proteins between cells by utilizing a contractile phage sheath-like structure (Leiman et al., 2009; Basler et al., 2012). Moreover, there is increasing evidence that the T6SS has important roles in the interaction with eukaryotic hosts as well as with other bacteria (Bingle et al., 2008; Cascales, 2008; Filloux et al., 2008; Basler et al., 2013). Mutations in the T6SS of the potato pathogen *Pectobacterium atrosepticum* led to increased virulence (Mattinen et al., 2008), whereas

T6SS-mutations in the endophyte *Azoarcus* sp. strain BH72 resulted in enhanced colonization of rice plants (Shidore et al., 2012). Similarly, inactivation of T6SS of *Rhizobium leguminosarum* enabled a strain that is normally not able to form functional nodules on pea to infect pea plants and fix nitrogen (Bladergroen et al., 2003). It is therefore possible that the T6SS elicits a host defence response and therefore it will be important to repress its expression in a beneficial endophyte.

It was observed that genes involved in flagella biosynthesis are mostly being down-regulated, however genes involved in chemotaxis are up-regulated in the presence of rice macerate. Chemotaxis is the mechanism through which bacteria are able to efficiently respond to changes in the chemical composition of the environment, moving towards favourable environments and avoiding unfavourable ones by controlling the flagellar movement. However, flagellins are known to elicit defence responses in plants as they are recognized as pathogen-associated molecular patterns (PAMPs) (Felix et al., 1999). Hence, the down-regulation of genes involved in flagellin synthesis would allow *B. kururiensis* to escape host defence responses. This has been observed in transcriptome experiments with *P. aeruginosa* PAO1 and *Azoarcus* sp. strain BH72 in response to root exudates (Mark et al., 2005; Shidore et al., 2012). Similarly, genes probably involved in root surface colonization (*e.g.* encoding type IV pili) are also differentially regulated in the presence of rice macerate as well as genes encoding components of efflux systems. The differential regulation of type IV pili-encoding genes has been also reported in plant-associated bacteria grown in the presence of root exudates (Mark et al., 2005; Shidore et al., 2012). This type of pili was reported to be important for endophytic rice colonization, root attachment, twitching motility and biofilm formation (Dörr et al., 1998; O'Toole and Kolter, 1998b; Bohm et al., 2007; Shidore et al., 2012). Efflux pumps are transport proteins involved in the extrusion of toxic substrates into the external environment. Several of these pumps were shown to be differentially regulated in the presence of rice macerate, such as components of the RND efflux system, which has been reported to play an active role in the successful colonization of the apple tree by the phytopathogen *Erwinia amylovora* (Burse et al., 2004). The study of Burse and coworkers suggests that the AcrAB system of *E. amylovora* is induced *in planta* and is involved in the resistance towards phytoalexins (plant secondary metabolites with antimicrobial activity). Our results obtained with the

RNAseq experiments suggest that for endophytes this type of efflux system might be important in their survival against plant defenses, as they are highly up-regulated in the presence of plant macerate with fold changes as high as 97 (Table 4.3).

N-acyl homoserine lactone (AHL) quorum-sensing (QS) via the BraI/R system has been shown to play a role in endophytic colonization and plant-growth-promotion (PGP) in *B. kururiensis* M130 (Suárez-Moreno et al., 2010). The BraI/R QS system was found to be up-regulated in the presence of rice plant macerate in the RNAseq experiment. Transcriptome studies have been performed to identify the BraI/R regulon in other closely related species of plant beneficial *Burkholderia* spp. highlighting that it is species-specific and no core regulon is present (Coutinho et al., 2013b). Currently the BraI/R regulon of *B. kururiensis* M130 is unknown and it is likely that it regulates loci that play important roles in endophytism. In addition, the well-known BraI/R regulator, RsaL (Suárez-Moreno et al., 2008), was not shown to be differentially expressed in response to rice macerate, which indicates that a currently unidentified regulator(s) is most probably involved in the regulation of this QS system in response to plant macerate.

Cordeiro and coworkers (2013) used total plant extract and proteomics in order to identify genes important for the endophytic lifestyle of the *Herbaspirillum seropedicae* endophyte. They analysed differences in the proteome in the presence or absence of sugar cane extract and were able to identify 16 differentially expressed proteins, most of them being metabolism-related (Cordeiro et al., 2013). In *B. kururiensis* M130 we observed 6 orthologs out of the 16 reported in the proteomics study with fold-changes higher than 2 and FDR values lower than 0.01 in response to rice macerate (Table 4.4). Most of these loci display differential expression with a similar pattern as seen for *H. seropedicae*. Interestingly one of these is the FusA elongation factor G1 and *B. kururiensis* has 2 copies of this gene. One of the *fusA* genes is up-regulated like the one of *H. seropedicae* while the other is down-regulated. In addition, the RpsA 30s ribosomal subunit S1 is up-regulated in *H. seropedicae* and the corresponding gene down-regulated in *B. kururiensis*. The 30S ribosomal protein S1 (RpsA) is the largest ribosomal protein in the 30S subunit of *E. coli* ribosome, however

Table 4.4. Differentially expressed proteins/genes of the endophytes *H. seropedicae* SmR1 and *B. kururiensis* M130 grown in the presence of sugar cane or rice plant extract, respectively.

Gene ID *H. seropedicae*	Gene function	Fold change *H. seropedicae*[1]	Gene ID *B. kururiensis*[3]	Gene location			Blast		RNAseq	
				Contig	Start	Stop	Score (bits)	Expect-value	Fold Change	FDR value
Hsero_3276	DapA dihydrodipicolinate synthase protein	2.09	fig|984307.6.peg.470	7	543771	542884	175	5.0e-45	8.68	8.5e-13
Hsero_0111	FusA elongation factor G1	2.52	fig|984307.6.peg.5368	7	838255	840360	1144	0.0	-3.18	9.4e-09
			fig|984307.6.peg.756	8	18080	20182	1118	0.0	2.3	0.0
Hsero_0415	Bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	Absent[2]	fig|984307.6.peg.893	7	981193	982758	815	0.0	-2.16	2.3e-11
Hsero_3923	Glutamate synthase small subunit oxidoreductase	Absent	fig|984307.6.peg.5425	8	67839	69305	801	0.0	-2.61	5.5e-16
Hsero_3689	RpsA 30s ribosomal protein S1	2.58	fig|984307.6.peg.3690	5	173542	175272	934	0.0	-2.34	2.7e-08
Hsero_0097	TufB EF-Tu elongation factor protein	Absent	fig|984307.6.peg.5356	8	1002	2192	736	0.0	-2.68	4.3e-66

[1] Fold change according to Cordeiro et al., 2013; [2] Protein absent in the presence of sugar cane extract; [3]Gene identity according to annotation performed by the RAST-Server (Aziz et al., 2008).

its association with the ribosome is weak and reversible (Subramanian, 1983). Moreover, RpsA is the only ribosomal protein with documented affinity for mRNA (Draper et al., 1977) and is essential for growth being required for translation of bulk mRNA in *E. coli* (Sorensen et al., 1998; Briani et al., 2008). For these reasons, RpsA has been considered a translation factor rather than a ribosomal protein. The reason for differential regulation of this protein upon exposure to plant macerate is currently unknown. Interestingly, RpsA expression has also been shown to be regulated in other plant-bacteria associations, for instance in the sugar cane associated bacterium *Gluconacetobacter diazotrophicus* (Lery et al., 2008; dos Santos et al., 2010)

EF-Tu is one of the most abundant proteins in bacteria and it plays an essential role in the elongation phase of protein biosynthesis. However EF-Tu, just like flagellin, is also recognized by the plant as a PAMP, activating the plant innate immune response (Kunze et al., 2004). These results suggest that the down-regulation of EF-Tu and flagellin by plant macerate is part of a coordinated regulation of proteins able to elicit plant defense mechanisms to enable temporal control of plant response and allow plant colonization by the endophyte.

In our genetic screen we have identified potential antisense RNAs (asRNAs) that are differentially expressed in the presence of rice plant extract. A similar methodology/approach was used in the identification of asRNAs of *Mycoplasma genitalium*, proving to be a robust technique for this type of analysis (Lluch-Senar et al., 2007). The RNAseq experiments further confirmed the transcription of these asRNAs and revealed that for some of them, the sense mRNA is also differentially expressed in the presence of plant macerate (Table 4.2). The presence of antisense transcripts has been linked to the regulation of gene expression in bacteria. AsRNAs are encoded on the DNA strand opposite another gene and are able to form extensive base pairing interactions with its corresponding sense RNA. This type of ncRNAs have been shown to have roles on the repression of transposases and toxic protein synthesis, regulation of the levels of transcriptional regulators, and modulation of the levels of metabolic and virulence proteins (Thomason and Storz, 2010). It is likely that the expression level of an asRNA is coupled to the expression level of its corresponding mRNA and evidence is accumulating that some asRNA respond to environmental cues (Georg and Hess, 2011). For instance, the transcription of *as_mccA*, an asRNA from *Clostridium*

acetobutylicum that controls a sulphur metabolic operon, is regulated in response to sulphur availability (Andre et al., 2008). It was not the scope of this work to focus on asRNAs, however recent research has evidenced that they are widespread in bacteria being found also in plant pathogens (Filiatrault et al., 2010; Georg and Hess, 2011; Schmidtke et al., 2012); currently to our knowledge there are no studies on their role in plant-bacteria interactions.

The reason(s) for the lack of RNA reads in the direction of the *gusA* gene in transposon insertions 36 and 66 is unknown, however from the β-glucuronidase quantification results (Appendix Table 7.10) it is possible to observe that these loci have a low transcription rate. Moreover, the RNA isolation procedure used here does not efficiently purify low molecular weight RNAs (*e.g.* small RNAs), therefore it is likely that we did not detect a complete representation of ncRNAs expressed under the conditions evaluated here.

In this study we have identified genes potentially regulated during the endophytic establishment of *B. kururiensis* M130 in rice plants. It is likely that endophytic bacteria utilize different strategies to interact with their hosts, however some of these features such as the differential regulation of molecules that elicit plant immune responses and those involved in root attachment are likely to be common. It is important to understand the mechanisms of regulation of these loci and the molecules/signals of the plant involved in inducing major changes in the bacterial behaviour required for *in planta* adaptation. A careful analysis of the transcriptional regulators and of the potential ncRNAs being differentially expressed in the presence of plant macerate is an important step in addressing these questions.

5 Summarizing Discussion

5.1 Main scope of this work

Burkholderia species are known for their ubiquity and versatile metabolism necessitating extensive regulatory mechanisms to support their fitness in many different environments. The new group of plant-beneficial and environmental (PBE) *Burkholderia* has emerged in the last 20 years and is considered a group of species with biotechnological potential. However, the molecular mechanisms involved in their interactions with the host/environment are currently not understood. In this work the responses of PBE *Burkholderia* to bacterial cell density changes and to macerated rice plant was studied. The major findings are summarized and discussed below.

5.2 The BraI/R QS system: molecules produced, regulon studies and its importance for plant-bacteria interactions

To better characterize the BraI/R N-acyl homoserine lactone quorum sensing (AHL-QS) system present in all species of the PBE *Burkholderia* group, the molecules produced by the BraI AHL synthase were identified in the following three species: the endophyte *B. phytofirmas* PsJN, the legume symbiont *B. phymatum* STM815 and the efficient biodegrader *B. xenovorans* LB400. The results of this analysis revealed that the system is responsible for the production of AHL molecules varying from 6 to 14 carbons with or without oxo or hydroxyl substitutions at the C-3 position, showing that the BraI orthologs in these species are able to direct biosynthesis of several different types of AHLs. However, the types of AHL molecules produced were different according to the medium used (*i.e.* rich and poor defined media), which is in agreement with studies showing that growth conditions can affect acyl-ACP (one of the precursors of AHLs) availability and consequently, the different production profile of AHLs (Brader et al., 2005). Moreover, through these and other experiments, *B. phymatum* STM815 was shown to produce larger amounts of AHL molecules than the other two species. The BraR protein in this species had a more promiscuous signal response compared to other BraR orthologs tested, and it responded to AHLs even at very low concentrations (*e.g.*10 nM). The significance of the promiscuous and sensitive response of BraR in *B. phymatum* is currently unknown. Does the system need to be on at lower cell-densities? Is *B. phymatum* eavesdropping on AHL produced by neighbours? What

is the role of the RsaL repressor in controlling or tempering this system? Future experiments will be directed towards obtaining answers to these questions. *In planta* studies are likely the most useful in approaching these questions.

The fact that the BraI/R system is present in all of the PBE *Burkholderia* spp. analysed suggests that it was originally present in their common ancestor (Suárez-Moreno et al., 2010). Thus, to verify if the system shares a common regulon amongst different species of *Burkholderia*, a transcriptome analysis of *B. phymatum* STM815 and *B. xenovorans* LB400 was performed. These experiments revealed that the QS system is responsible for the regulation of 4% of the protein-encoding genes of *B. xenovorans* LB400 and 2.3% of *B. phymatum* STM815. Interestingly, most of the differentially regulated genes of strain LB400 were up-regulated in wild type compared to QS mutant, whereas in strain STM815 they were down-regulated in wild type compared to QS mutant. The BraI/R QS system is therefore responsible for repression of many genes in strain STM815 whereas it is involved in activation of several genes in LB400 . This is a significant difference among the two species which merits attention. The BraI/R of *B. xenovorans* LB400 is involved in the regulation of a large number of genes encoding ABC transporter components and genes involved in the degradation of aromatic compounds, while in *B. phymatum* STM815 it is involved in the regulation of DNA/RNA modification enzymes, genes related to DNA repair and recombination and of components of the membrane/cell wall or of enzymes important for their synthesis.

The comparison of the two regulons revealed that only four common genes were regulated in the two species of PBE *Burkholderia*, which indicates a unique role for BraI/R in each species. Interestingly, two of these four genes were related to EPS production and were part of the cepacian biosynthesis cluster. Further experiments with other PBE *Burkholderia* species suggested that the QS system is involved in the regulation of EPS biosynthesis in all the PBE species. The importance of EPS for both plant and soil bacteria is well known serving as a protection mechanism and/or as the first contact between the bacteria and its host (D'Haeze et al., 2004) and the regulation of EPS production is a common phenotype that is regulated by cell-density in this group of bacteria. Further validations of the BraI/R regulon in two species of the PBE group that occupy similar niches would provide further insights into the role of this QS system in this group of *Burkholderia*.

In planta experiments revealed that the BraI/R system is not important for nodulation and plant growth promotion of *M. pudica* by *B. phymatum* STM815. Furthermore it is not involved in endophytic colonization and plant growth promotion of maize by *B. phytofirmas* PsJN. Therefore, it does not play a role *in planta* under the conditions tested here. Studies on *in planta* localization and bacterial community structures are necessary in order to understand the lifestyle of these bacteria and to determine if QS is an important trait. Moreover, some of the PBE *Burkholderia* carry another AHL-based and/or a BDSF-based QS system (Suárez-Moreno et al., 2010; Deng et al., 2012), thus it is possible that these other systems are involved in plant-bacteria interactions or endophytic colonization. For example, the XenI2/R2 of *B. phytofirmas* PsJN was reported to be important for endophytic colonization and plant growth promotion of *Arabidopsis thaliana* (Zúñiga et al., 2013).

5.3 *Burkholderia kururiensis* M130: from genome to transcriptome

In order to obtain more information on the rice endophyte *B. kururiensis* M130 and to better understand its ability to colonize and promote plant growth, its genome was sequenced and analysed. This study revealed that this bacterium possesses a genome of ~7.1 Mb, which is comparable to the size of previously sequenced *Burkholderia* spp. of the PBE group (Suárez-Moreno et al., 2012). Several genes potentially involved in adherence and endophytic colonization of plant roots were identified such as those involved in the production of flagella and type IV pili (Bohm et al., 2007; Buschart et al., 2012). Phenotypes encoded by the genome which are likely to be responsible for the plant growth promotion ability of strain M130 include nitrogen fixation, as it possesses a complete cluster responsible for this phenotype that is very similar to the one of the maize endophyte *B. unamae* MTI-641, and the production of ACC deaminase enzyme which degrades the phytohormone ethylene, protecting the plant from its stress-induced deleterious effects. In addition, genes involved in the production of the plant hormone IAA are present, however, no complete pathway for its production was identified. Thus, it is still unknown whether *B. kururiensis* M130 is able to produce IAA.

The M130 genome possesses a variety of features that might help the bacteria survive inside the plant and overcome its defenses; for instance, several genes involved in the production of peroxidases, catalases and other enzymes that can act against ROS. In addition, several RND multidrug efflux pumps are present which could be involved in the resistance to plant toxic molecules, as it was shown for the phytopathogen *E. amylovora* (Burse et al., 2004). The genome of strain M130 also encodes a large number of dioxygenases that are responsible for the ring cleavage of organic aromatic compounds and might protect bacteria against plant-derived toxic compounds. Another interesting feature is the presence of the different types of secretion systems, with the exception of T1SS and T4SS. The importance of different types of secretion systems for endophytic bacteria is not known, however, it is believed that some of them might have an important role in plant-bacteria interactions. The T6SS, for instance, was shown to be very common among this type of bacteria (Reinhold-Hurek and Hurek, 2011; Mitter et al., 2013) and was abundantly represented in a metagenome of rice root endophytes (Sessitsch et al., 2012). Although the features analyzed in this work are not common to all bacterial endophytes (Mitter et al., 2013), their possible role in endophytic colonization needs attention.

With the intent of further investigating the features of *B. kururiensis* M130 that are important for its interaction with the rice plant, experiments were designed to assess global changes in gene expression in response to the plant environment. Two approaches were used; firstly, a more traditional screen of a transposon promoter-probe mutant genome bank and secondly via a genome-wide scale RNAseq technique. These experiments revealed that *B. kururiensis* M130 undergoes major gene expression changes when exposed to plant macerate, most notably in metabolic functions probably as a result of the availability of a new range of nutrients. In addition, differential expression of several loci encoding for families of transporter proteins that are probably involved in the transport of these new nutrients and plant molecules, was also observed.

Several of the features possibly involved in plant-bacteria interactions, previously identified via genome mining were shown to be differentially regulated in the presence of plant macerate. For example, the down-regulation of genes involved in flagella biosynthesis and T6SS might help bacteria avoid the plant immune system as both of these features are believed to elicit a local host defence (Felix et al., 1999;

Mattinen et al., 2008; Shidore et al., 2012). In addition, up-regulation of genes encoding RND multidrug efflux pumps, which are believed to help the bacteria to survive against phytoalexins was also observed (Burse et al., 2004). Moreover, the differential regulation of genes involved in the production of type IV pili was also detected, and these molecules might be important for plant colonization (Shidore et al., 2012). Another interesting result from these experiments was the up-regulation of the AHL QS system BraI/R, which has been demonstrated to be important for endophytic colonization of rice by *B. kururiensis* M130 (Suárez-Moreno et al., 2010). Deciphering the BraI/R regulon in the presence of plant macerate will help to elucidate its role *in planta.*

The data from the transposon promoter-probe screening combined with the one of the RNAseq experiment allowed the identification of putative antisense RNAs (asRNAs) that might be involved in plant-bacteria interactions. Currently, there are no studies on the importance of asRNAs in endophytic bacteria, but there is new evidence that these molecules are widespread amongst bacteria and are involved in the regulation of many different phenotypes (Thomason and Storz, 2010). For this reason, analysis of regulatory functions of asRNAs will most likely be subject of intense studies in the future.

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7 Appendix

7.1 Tables

Table 7.1. Primers used for cloning purposes. Restriction sites are underlined.

Plasmids	Primer name	Primer sequence	Amplicon size (bp)
pKNOCK plasmids			
pKNOCK- $BRAI_{PsJN}$	Phytof.luxI2.F	5'- AGATCGACGGCTATGACGC -3'	353
	Phytof.luxI2.R	5'- GCTGATGTCGATGCCGGTT -3'	
pKNOCK- $BRAI_{phym}$	Phymat.luxI.F	5'- ATGCTCATTCAGGACGGCGAT -3'	246
	Phymat.luxI.R	5'- AATGCCCATGCGGTCTGCGA -3'	
pBBR1MCS-3 derivatives			
$pBBRbraI_{PsJN}$	Phytof.braI.F	5'- GGTTTGCGGTTGCCAATGA -3'	834
	Phytof.braI.R	5'- TAACAGCCAGGCAGCAAGCA -3	
$pBBRbraI_{phym}$	Phymat.braI.F	5'- TTCTTGCGGTACGCGATGA -3'	919
	Phymat.braI.R	5'- GCCGGATATCGCATATCACG -3'	
pMP220 derivatives			
$pMPbraI_p$	PhymbraI.promF	5'- CAGAATTCAAGTTCCATGATGTTTTCC -3'	203
	PhymbraI.promR	5'- AAGGTACCATGTGGTTGTTGTCGAA -3'	
$pMPeps_p$	PhymEPS.promF	5'- CAGAATTCGAATTTTAATGCCGCTTTATT GAGCCTT -3'	189
	PhymEPS.promR	5'- AACTGCAGGCCTCGTCTGTCAGTCCAA -3'	

Table 7.2. Primers used in the SQ RT-PCR experiments.

Gene ID	Primer name	Primer sequence	Amplicon size (bp)
B. phymatum			
Bphy_R0017	Bphy_R0017-F	5'- CAACCCTGATCCAGCAATGC -3'	288
	Bphy_R0017-R	5'- CCCTCTGCCATACTCTAGCC -3'	
Bphy_1266	Bphy_1266-F	5'- ACAAGGTCTCGCTGGGTAC -3'	238
	Bphy_1266-R	5'- AAGATGGAGCGGGTGTACTC -3'	
Bphy_0385	Bphy_0385-F	5'- CAGTGGCACGAAGATTTTCATCTC -3'	256
	Bphy_0385-R	5'- CCATCCCTTCGCATTGTCG -3'	
Bphy_1473	Bphy_1473-F	5'- CAATACAAGGGCGACGACG -3'	268
	Bphy_1473-R	5'- GCTGGATCTGGATCGTGTTC -3'	
Bphy_1029	Bphy_1029-F	5'- GACCTCGACATTCCGCATG -3'	267
	Bphy_1029-R	5'- GAGCGAACCGTTCAGATCGA -3'	
Bphy_0444	Bphy_0444-F	5'- GTCAAGGAAGCGAAGGCG -3'	250
	Bphy_0444-R	5'- TGATGCCGATCTTCAGTTCGTC -3'	
Bphy_0250	Bphy_0250-F	5'- GCGGGAGTGGAAAGTAACAAA -3'	234
	Bphy_0250-R	5'- GCTTAACTGTGCCTCAGTGAGA -3'	
Bphy_1089	Bphy_1089-F	5'- GCGTACTGCTCGTTCTCGTA -3'	235
	Bphy_1089-R	5'- GGCATCGTGTTGTCCCTTG -3'	
Bphy_1325	Bphy_1325-F	5'- CGGTCGCATCAAGAAGATCG -3'	273
	Bphy_1325-R	5'- TGCAACATCAGTTCCATCGC -3'	
B. xenovorans			
Bxe_BR0001	16S_BXE_FW	5'- GGCGCAAGCCTGATCCAGCA -3'	237
	16S_BXE_RV	5'- TCTTAGCGAACCGCCTGCGC -3'	
Bxe_B0689	Bxe_B0689-F	5'- CTTGCTCAGCCTCGTGACA -3'	268
	Bxe_B0689-R	5'- GCTGATGAAGAAACTGCGATC -3'	
Bxe_B0692	Bxe_B0692-F	5'- ACTGGATCAAGCCGCTGTTT -3'	270
	Bxe_B0692-R	5'- TGATGATGAGCGGCAAATAGG -3'	
Bxe_B0684	Bxe_B0684-F	5'- TTCCGATGCGTTCAACCGT -3'	285
	Bxe_B0684-R	5'- AACTCGAACAGGTCGCAAC -3'	
Bxe_A0141	Bxe_A0141-F	5'- CCAAGCAGGTCGTGTTCG -3'	256
	Bxe_A0141-R	5'- TTGGCGGTGTTCAAGGTCT -3'	
Bxe_A1431	Bxe_A1431-F	5'- GGAAAGCGTCTTCGGACTA -3'	262
	Bxe_A1431-R	5'- CCTTTCGCCAGCAACTGA -3'	
Bxe_B0892	Bxe_B0892-F	5'- GTCTTCGGACTACGGCTTTC -3'	260
	Bxe_B0892-R	5'- TAAAGCCTTTCGCCAGCAAC -3'	
Bxe_B1584	Bxe_B1584-F	5'- CTACAACACGCAGAACCTCG -3'	249
	Bxe_B1584-R	5'- GCGATCTCTTTCAGTTGCTCG -3'	
Bxe_B1166	Bxe_B1166-F	5'- GAGGATTCCCTTATGCAGATCAC -3'	263
	Bxe_B1166-R	5'- CTATGCAACACTACGCCTTCTC -3'	

Table 7.3. Identification of the different AHLs produced by the BraI/R system of *B. phymatum* STM815, *B. phytofirmas* PsJN and *B. xenovorans* LB400 and by the XenI2/R2 system of *B. xenovorans* LB400 in two different media.

		Analyte Peak Areas																	
		AHLs						3-oxo-HSL						3-hydroxy-HSL					
		C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}
Media	Sample\ *m/z*	172.1/ 102.1	200.1/ 102.1	228.1/ 102.1	256.1/ 102.1	284.1/ 102.1	312.1/ 102.1	186.1/ 102.1	214.1/ 102.1	242.1/ 102.1	270.1/ 102.1	298.1/ 102.1	326.2/ 102.1	188.1/ 102.1	216.1/ 102.1	244.1/ 102.1	272.1/ 102.1	300.1/ 102.1	328.2/ 102.1
KB	STM815[a]	0	29000	7.6E+5	1.6E+6	1.9E+5	46300	0	2.3E+5	4.6E+6	2.4E+7	1.8E+6	1.2E+5	0	56900	2.3E+6	2.7E+7	7.4E+6	1.7E+5
KB	STM815BRAI[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M9-glu	STM815[a]	0	28400	1.4E+5	2.1E+5	18200	5090	0	91800	1.7E+6	4.5E+6	4.4E+5	25000	0	37600	4.8E+5	1.7E+6	1.3E+5	0
M9-glu	STM815BRAI[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KB	PsJN[b]	0	0	19200	0	11400	62200	0	0	0	4440	36800	5.9E+5	0	0	4.8E+5	9070	36800	1.8E+6
KB	PsJNBRAI[b]	0	0	15200	0	0	0	0	0	0	0	0	0	0	0	3.9E+5	3940	0	0
M9-glu	PsJN[b]	0	12700	1.2E+5	4790	40300	1.3E+5	0	0	4560	25800	1.1E+5	2.7E+5	0	8830	1.2E+6	62700	5.0E+5	3.9E+6
M9-glu	PsJNBRAI[b]	0	9710	95380	0	0	0	0	0	0	0	0	0	0	7460	8.8E+5	15400	0	0
KB	LB400[b]	0	0	16100	0	34700	3.9E+5	0	0	19200	51600	2.7E+5	4.4E+6	0	0	3.1E+5	15600	1.7E+5	6.8E+6
KB	LB400BRAI[b]	0	0	8500	0	0	0	0	0	0	0	0	0	0	0	2.4E+5	2390	0	0
KB	LB400XENI2[b]	0	0	13600	0	51700	4.4E+5	0	0	35400	91100	3.9E+5	3.6E+6	0	0	13800	20700	4.1E+5	9.1E+6
M9-glu	LB400[b]	0	11300	70380	0	35500	1.6E+5	0	0	6590	16200	58200	2.8E+5	0	0	4.9E+5	29000	1.1E+5	4.2E+6
M9-glu	LB400BRAI [b]	0	6130	21280	0	0	0	0	0	0	0	0	0	0	0	1.7E+5	6680	0	0
M9-glu	LB400XENI2[b]	7	9410	9480	0	34200	1.1E+5	0	0	9530	21100	86100	2.1E+5	0	0	12200	15400	2.8E+5	6.3E+6
KB	Media Blank	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M9-glu	Media Blank	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ethyl acet.	Solvent Blank	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synth. Std.	Posit. Control	2.4E+6	2.5E+6	7.6E+5	2.7E+5	1.1E+6	7.2E+5	2.7E+6	3.1E+6	2.0E+6	1.6E+6	60600	8.5E+5	2.6E+6	1.7E+6	3.6E+6	3.6E+6	1.1E+5	8.3E+5

KB, King's B medium; M9-glu, M9 minimal medium supplemented with glucose and casamino acids

[a]AHL extraction from cell-free spent supernatant from a culture grown overnight in 20 ml of medium

[b]AHL extraction from cell-free spent supernatant from a culture grown overnight in 50 ml of medium

Table 7.4. Validation by semi-quantitative reverse transcription-polymerase chain reaction (SQ RT-PCR) of various differentially expressed genes.

Gene ID	Gene description	Microarray fold difference	SQ RT-PCR fold difference[a]
***B. xenovorans* LB400**			
Bxe_B0689	Conserved hypothetical protein	-4.05	-17.5 ± 3.2
Bxe_B0692	ABC taurine transporter, inner membrane subunit	-3.09	-2.7 ± 0.8
Bxe_B0684	Putative taurine dehydrogenase, small subunit	-2.89	-20.5 ± 8.3
Bxe_A0141	Flagellar basal-body rod protein FlgC	-2.75	-2.9 ± 0.5
Bxe_B1584	Membrane dipeptidase	3.69	22.2 ± 6.1
Bxe_B0892	ABC sugar transporter, ATPase subunit	4.40	12.8 ± 1.2
Bxe_B1166	Conserved hypothetical protein	4.67	17.5 ± 2.6
Bxe_A1431	Putative dihydropyrimidinase	5.44	14.4 ± 1.7
***B. phymatum* STM815**			
Bphy_0444	Succinyl-CoA synthetase, alpha subunit	-6.39	-7.7 ± 1.3
Bphy_0250	Conserved hypothetical protein	-6.37	-2.0 ± 0.07
Bphy_1089	Arginine/ornithine antiporter	-3.70	-2.5 ± 0.03
Bphy_1325	Lipid-A-disaccharide synthase	-3.54	-1.9 ± 0.1
Bphy_1029	Nicotinamidase	4.37	1.9 ± 0.2
Bphy_1473	Conserved hypothetical protein	9.68	18.0 ± 2.9
Bphy_0385	Acyl-CoA dehydrogenase domain protein	10.38	7.0 ± 0.6
Bphy_1266	Acriflavin resistance protein	14.13	4.1 ± 1.4

[a]Mean from three independent RNA isolations ± standard deviation (SD).

Table 7.5. BraI/R regulon in *B. xenovorans* LB400.

Gene ID	Gene product	Fold change[a]
Hypothetical proteins		
Bxe_B0689	Conserved hypothetical protein	-4.05
Bxe_A3822	Conserved hypothetical protein	-1.94
Bxe_B0793	Conserved hypothetical protein	-1.84
Bxe_A4465	Conserved hypothetical protein	-1.78
Bxe_A2661	Hypothetical protein	-1.74
Bxe_B0810	Conserved hypothetical protein	-1.72
Bxe_A1112	Conserved hypothetical protein	-1.71
Bxe_B2781	Hypothetical protein	-1.70
Bxe_A1114	Conserved hypothetical protein	-1.65
Bxe_A1932	Conserved hypothetical protein	-1.64
Bxe_A0251[b]	Hypothetical protein	-1.64
Bxe_B2914	Conserved hypothetical protein	-1.60
Bxe_A1538	Conserved hypothetical protein	-1.59
Bxe_B0045[b]	Hypothetical protein	-1.59
Bxe_B2913[b]	Hypothetical protein	-1.58
Bxe_A0079	Conserved hypothetical protein	-1.53
Bxe_B0120	Conserved hypothetical protein	-1.52
Bxe_B0044[b]	Hypothetical protein	-1.52
Bxe_B0046[b]	Hypothetical protein	-1.52
Bxe_C0724	Conserved hypothetical protein	-1.51
Bxe_B0813[b]	Hypothetical protein	-1.51
Bxe_B0387	Conserved hypothetical protein	-1.50
Bxe_A2463	Conserved hypothetical protein	1.50
Bxe_B1891	Conserved hypothetical protein	1.50
Bxe_B2681[b]	Hypothetical protein	1.51
Bxe_B0891	Conserved hypothetical protein	2.52
Bxe_C0464	Conserved hypothetical protein	1.53
Bxe_C0647	Conserved hypothetical protein	1.53
Bxe_C0026[b]	Hypothetical protein	1.54
Bxe_A0831	Hypothetical protein	1.54
Bxe_C0185	Putative membrane protein	1.55
Bxe_B1076	Conserved hypothetical protein	1.55
Bxe_B0487	Hypothetical protein	1.56
Bxe_B2168	Conserved hypothetical protein	1.57
Bxe_A2944	Conserved hypothetical protein	1.57
Bxe_C0962	Conserved hypothetical protein	1.58
Bxe_A3306	Hypothetical protein	1.59
Bxe_A0702	Conserved hypothetical protein	1.59
Bxe_A3378	Conserved hypothetical protein	1.59

Bxe_A4442	Conserved hypothetical protein	1.60
Bxe_B1791	Conserved hypothetical protein	1.60
Bxe_A2030	Conserved hypothetical protein	1.61
Bxe_B2220	Conserved hypothetical protein	1.61
Bxe_C0027	Hypothetical protein	1.61
Bxe_A2271	Hypothetical protein	1.62
Bxe_B2012	Hypothetical protein	1.63
Bxe_A0642	Conserved hypothetical protein	1.64
Bxe_B1854	Hypothetical protein	1.64
Bxe_C0184	Conserved hypothetical protein	1.66
Bxe_C0206	Conserved hypothetical protein	1.66
Bxe_A2192	Conserved hypothetical protein	1.68
Bxe_B1280	Conserved hypothetical protein	1.68
Bxe_B0226	Hypothetical protein	1.68
Bxe_B0005	Conserved hypothetical protein	1.69
Bxe_B1167	Conserved hypothetical protein	1.70
Bxe_B2455	Conserved hypothetical protein	1.70
Bxe_B1019	Hypothetical protein	1.74
Bxe_C0186	Conserved hypothetical protein	1.74
Bxe_A2226	Hypothetical protein	1.74
Bxe_A3672	Conserved hypothetical protein	1.74
Bxe_C1021	Conserved hypothetical protein	1.78
Bxe_C1287	Conserved hypothetical protein	1.81
Bxe_B1081	Putative membrane protein	1.81
Bxe_A2025	Conserved hypothetical protein	1.83
Bxe_C0075	Conserved hypothetical protein	1.85
Bxe_C1024	Conserved hypothetical protein	1.86
Bxe_A1288	Conserved hypothetical protein	1.87
Bxe_B1171[b]	Hypothetical protein	1.90
Bxe_A2236	Conserved hypothetical protein	1.97
Bxe_B0024	Conserved hypothetical protein	2.00
Bxe_A1425	Conserved hypothetical protein	2.03
Bxe_A1457	Conserved hypothetical protein	2.04
Bxe_B1214	Conserved hypothetical protein	2.10
Bxe_A4478	Hypothetical protein	2.14
Bxe_C0169	Conserved hypothetical protein	2.15
Bxe_B0317	Conserved hypothetical protein	2.19
Bxe_A1453	Putative lipoprotein	2.24
Bxe_A1455	Conserved hypothetical protein	2.25
Bxe_A1454[b]	Hypothetical protein	2.26
Bxe_B1165	Conserved hypothetical protein	2.33
Bxe_B0669	Conserved hypothetical protein	2.34
Bxe_A4366	Conserved hypothetical protein	2.38

Bxe_A2327	Conserved hypothetical protein	2.37
Bxe_A3351	Conserved hypothetical protein	2.51
Bxe_B1395	Conserved hypothetical protein	2.68
Bxe_A2977	Putative membrane protein	2.71
Bxe_B0936	Conserved hypothetical protein	2.94
Bxe_A3352	Conserved hypothetical protein	3.14
Bxe_B1169	Conserved hypothetical protein	3.19
Bxe_B1331	Hypothetical protein	3.51
Bxe_B0559	Conserved hypothetical protein	3.79
Bxe_C0165	Conserved hypothetical protein	3.79
Bxe_B1160	Conserved hypothetical protein	4.30
Bxe_B1166	Conserved hypothetical protein	4.67
	Total	**94**
Trancriptional regulators		
Bxe_A2662	Phage Transcriptional regulator, AlpA	-1.93
Bxe_B1778	Transcriptional regulator, TetR family	-1.50
Bxe_B1586	Transcriptional regulator, AraC family	1.51
Bxe_A4440	Transcriptional Regulator, AraC family	1.51
Bxe_B0944	Transcriptional regulator, XRE family	1.52
Bxe_A2061	Putative cold shock transcriptional regulator,	1.54
Bxe_A0913	Putative cold-shock DNA-binding domain protein	1.56
Bxe_B0309	Fis family GAF modulated sigma-54 specific transcriptional regulator	1.56
Bxe_B2442	Transcriptional regulator, AraC family	1.58
Bxe_B2439	Sigma-54 dependent transcriptional regulator	1.66
Bxe_B0280	Transcriptional regulator, MarR family	1.75
Bxe_B2151	Transcriptional regulator, LysR family	1.79
Bxe_C0963	Transcriptional regulator, LysR family	1.94
Bxe_B0310	Sigma54 specific transcriptional regulator with	1.96
Bxe_A1426	Transcriptional regulator, TetR family	2.12
Bxe_B0609[c]	Transcriptional regulator, RsaL	3.83
	Total	**16**
ABC transporters		
Bxe_B0692	ABC taurine transporter, inner membrane subunit	-3.09
Bxe_B0694	ABC taurine transporter, periplasmic ligand binding protein	-2.20
Bxe_B0693	ABC taurine transporter, ATPase subunit	-1.80
Bxe_B2995	ABC glycine betaine/choline/proline family	-1.59
Bxe_A0546	ABC sugar(arabinose) transporter, periplasmic subunit	1.51
Bxe_A1432	ABC polar amino acid transporter, periplasmic subunit	1.58
Bxe_C1003	ABC branched chain amino acid family	1.57
Bxe_A1328	ABC sugar transporter, inner membrane subunit	1.60
Bxe_B1826	ABC amino acid transporter, ATPase subunit	1.65

Bxe_B0590	ABC sugar transporter, periplasmic ligand binding protein	1.66
Bxe_B0456	ABC nitrate/sulfonate/bicarbonate family	1.68
Bxe_A3706	ABC branched chain amino acid family	1.71
Bxe_B1405	ABC sugar transporter, inner membrane subunit	1.73
Bxe_B0893	ABC sugar transporter, inner membrane subunit	1.74
Bxe_A0020	ABC branched chain amino acid family	1.74
Bxe_A0017	ABC branched chain amino acid family	1.75
Bxe_A2191	ABC sugar transporter, periplasmic ligand binding protein	1.78
Bxe_A1327	ABC sugar transporter, ATPase subunit	1.82
Bxe_B2964	ABC sugar transporter, periplasmic ligand binding protein	1.83
Bxe_B2963	ABC sugar transporter, ATPase subunit	1.84
Bxe_A1434	ABC polar amino acid transporter, ATPase subunit	1.87
Bxe_A0021	ABC branched chain amino acid family	1.88
Bxe_B1397	ABC sugar transporter, inner membrane subunit	1.92
Bxe_A0018	ABC branched chain amino acid family	1.95
Bxe_A0019	ABC branched-chain amino acid family	1.98
Bxe_C1271	ABC sugar transporter, inner membrane subunit	2.11
Bxe_A1329	ABC sugar transporter, periplasmic ligand binding protein	2.13
Bxe_B1576	ABC proline/glycine betaine transporter, periplasmic ligand binding protein	2.23
Bxe_B1404	ABC sugar transporter, ATPase subunit	2.25
Bxe_A3673	ABC sugar transporter, periplasmic ligand binding protein	2.26
Bxe_B0457	ABC nitrate/sulfonate/bicarbonate family	2.28
Bxe_B1402	ABC sugar transporter, inner membrane subunit	2.28
Bxe_C1270	ABC sugar transporter, periplasmic ligand binding protein	2.32
Bxe_B1616	ABC proline/glycine betaine transporter, ATPase	2.34
Bxe_B0455	ABC nitrate/sulfonate/bicarbonate family	2.47
Bxe_B0940	ABC putrescine transporter, periplasmic ligand binding protein	2.50
Bxe_C1269	ABC sugar transporter, fused ATPase subunits	2.62
Bxe_B1403	ABC sugar transporter, periplasmic ligand binding protein	2.77
Bxe_B0894	ABC sugar transporter, periplasmic ligand binding protein	2.90
Bxe_B1615	ABC proline/glycine betaine transporter, inner membrane subunit	3.00
Bxe_B1396	ABC sugar transporter, periplasmic ligand binding protein	3.25
Bxe_B0892	ABC sugar transporter, ATPase subunit	4.40
	Total	**42**
Membrane/ Other transporters		
Bxe_A0141	Flagellar basal-body rod protein FlgC	-2.75
Bxe_B0686	Beta-alanine/gamma-aminobutyrate-H+ symporter	-2.33
Bxe_A0143	Putative flagellar hook protein FlgE	-1.75
Bxe_A1509	Putative proline/betaine major facilitator	-1.73
Bxe_A0145	FlgG; flagellar basal-body rod protein	-1.67

Bxe_B0794	TonB-dependent siderophore receptor	-1.64
Bxe_B0531	TonB-dependent siderophore receptor	-1.59
Bxe_A2798	Putative outer membrane channel signal peptide	1.52
Bxe_C0243	Major facilitator superfamily (MFS)metabolite/H+	1.61
Bxe_C1273	Sugar binding or transport, RbsD/FucU	1.63
Bxe_C1231	Outer membrane porin, OmpC family	1.71
Bxe_A1430	Nucleobase/cation symporter, NCS1 family	1.71
Bxe_A2797	Putative pilus assembly protein, CpaE-like	1.77
Bxe_C0698	Outer membrane porin, OmpC family	1.78
Bxe_A0668	Major facilitator superfamily (MFS) transporter	1.78
Bxe_C1012	Outer membrane porin, OmpC family	1.87
Bxe_A2802	Putative pilus subunit protein, PilA like	1.96
Bxe_A3224	Sulphate transporter	2.01
Bxe_A4445	Ethanolamine transporter, EAT family, APC	2.19
Bxe_B0285	Major facilitator superfamily (MFS) sugar	2.24
Bxe_A2321	Putative transmembrane protein, AsmA-like	2.38
Bxe_A2793	Putative lipoprotein transmembrane	2.42
Bxe_B1170	Phospholipid binding protein	2.52
Bxe_A2796	Putative type II/IV secretion ATPase protein,	2.57
Bxe_B1584	Membrane dipeptidase	3.69
	Total	**25**
EPS production		
Bxe_A2237	Predicted glycosyl transferase, group 1	1.56
Bxe_A2239	Putative transmembrane protein	1.64
Bxe_A2242	Protein-tyrosine kinase	1.90
Bxe_A2238	Putative glycosyl transferase, group 1	2.41
Bxe_A2245	UDP-glucose 6-dehydrogenase	2.48
	Total	**5**
Aromatic compounds degradation		
Bxe_A1113	Putative dioxygenase	-1.51
Bxe_B2031	3,4-dihydroxyphenylacetate 2,3-dioxygenase	1.58
Bxe_B0274	Carboxymethylenebutenolidase	1.77
Bxe_B0277	Putative 4-hydroxybenzoyl CoA thioesterase	1.81
Bxe_B0278	Putative acyl-CoA dehydrogenase	1.88
Bxe_B0276	Putative aerobic 2-aminobenzoate	1.89
Bxe_B0279	Putative enoyl-CoA hydratase/isomerase	1.90
Bxe_B0273	Putative glutathione S-transferase	1.95
Bxe_C1285	Putative mandelate racemase/muconate	1.96
Bxe_B0589	Putative hydrolase	2.05
Bxe_B0275	Putative acid-coenzyme A ligase	2.14
Bxe_C1025	Homogentisate 1,2-dioxygenase (HmgA)	2.28
Bxe_C1022	Salicylate 1-monooxygenase	2.28

Bxe_C0895	Aldehyde dehydrogenase (box pathway)	2.47
Bxe_C0890	Benzoyl-CoA oxygenase component A (boxA)	2.58
Bxe_C0891	Benzoyl-CoA oxygenase component B (boxB)	2.85
Bxe_B1578	Iron-sulphur Rieske protein	3.05
Bxe_A1424	Benzoyl-CoA oxygenase component A (boxA)	3.25
Bxe_C0897	Putative lactonase (box pathway)	3.28
Bxe_C0892	Benzoyl-CoA-dihydrodiol lyase (boxC)	3.51
	Total	**20**
Cellular processes/ Metabolism		
Bxe_B0690	Phosphate acetyltransferase	-3.10
Bxe_B0684	Putative taurine dehydrogenase, small subunit	-2.89
Bxe_B0687	Putative cobalt insertion protein	-2.82
Bxe_B0685	Putative taurine dehydrogenase, large subunit	-2.45
Bxe_B1770	Response regulator receiver domain	-1.96
Bxe_B0792	Putative 2OG-Fe(II) oxygenase superfamily	-1.92
Bxe_B1637	Cytochrome o ubiquinol oxidase subunit II	-1.85
Bxe_B0691	Sulphoacetaldehyde acetyltransferase	-1.80
Bxe_B1636	Cytochrome-c oxidase	-1.67
Bxe_A0496	Arginine biosynthesis bifunctional protein,	-1.64
Bxe_A4136	Histidinol-phosphate aminotransferase	-1.59
Bxe_A2809	Putative ATPase, AFG1-like	-1.57
Bxe_A1652	Putative ATP-dependent RNA helicase	-1.56
Bxe_A1115	Putative dehydrogenase/oxidoreductase protein	-1.56
Bxe_A4135	Peptidyl-tRNA hydrolase	-1.56
Bxe_A1929	Ureidoglycolate hydrolase	-1.56
Bxe_B0832	Putative oxidoreductase	-1.54
Bxe_B1635	Cytochrome o ubiquinol oxidase subunit III	-1.53
Bxe_B0333	Phosphocarrier HPr protein	-1.51
Bxe_C0814	Putative short-chain dehydrogenase/reductase	-1.51
Bxe_B0118	Putative HesA/moeB/thiF family protein	-1.50
Bxe_C0922	Putative 3-hydroxyacyl-CoA dehydrogenase	1.50
Bxe_B1817	Putative oxidoreductase	1.50
Bxe_B2861	Putative alpha-amylase-related protein	1.51
Bxe_B2610	Histidine Kinase	1.51
Bxe_C0244	Methylmalonate-semialdehyde dehydrogenase	1.52
Bxe_B1311	PAS/PAC sensor hybrid histidine kinase	1.52
Bxe_B0963	Putative biotin carboxylase	1.52
Bxe_B2023	Methylmalonate-semialdehyde dehydrogenase	1.54
Bxe_A3891	Putative aromatic aminotransferase	1.53
Bxe_A1331	Myo-inositol 2-dehydrogenase	1.54
Bxe_A1326	Putative myo-inositol catabolism LolC protein	1.54
Bxe_A3570	Putative electron transfer flavoprotein beta-	1.54

Bxe_C0368	Putative periplasmic nitrate reductase	1.55
Bxe_C0245	3-hydroxyisobutyrate dehydrogenase	1.55
Bxe_B2221	ExodeoxyribonucleaseIII xth	1.56
Bxe_C0207	Putative acyl-CoA dehydrogenase	1.56
Bxe_B1268	Glutathione S-transferase	1.56
Bxe_B1589	Glutathione-independent formaldehyde	1.56
Bxe_A3573	Putative AMP-binding enzyme	1.57
Bxe_A4356	Putative carbon monoxide dehydrogenase, CoxG	1.57
Bxe_C1055	Putative fumarylacetoacetate (FAA) hydrolase	1.57
Bxe_B0716	Putative pyrimidine reductase	1.58
Bxe_A2879	Putative alcohol dehydrogenase,	1.58
Bxe_B2960	Altronate dehydratase	1.58
Bxe_A0965	NAD-dependent formate dehydrogenase, beta	1.59
Bxe_A1836	Putative pyruvate dehyrdogenase	1.59
Bxe_A4352	Carbon-monoxide dehydrogenase form II, large	1.60
Bxe_B0943	Putative glutamine amidotransferase	1.61
Bxe_A2248	Mannose-1-phosphateguanylyltransferase/mannose-	1.61
Bxe_C1017	Putative tripartite tricarboxylate	1.62
Bxe_A3575	3-hydroxyisobutyrate dehydrogenase	1.62
Bxe_A4444	Putative ethanolamine ammonia lyase large	1.63
Bxe_C0369	Putative periplasmic nitrate reductase	1.64
Bxe_B1825	Histidine ammonia-lyase	1.64
Bxe_A2943	Imidazolonepropionase	1.64
Bxe_A2942	Formiminoglutamate deiminase	1.65
Bxe_A1395	Putative acetyl-CoA synthetase	1.65
Bxe_A4353	Putative carbon monoxide dehydrogenase, medium	1.66
Bxe_A3374	Alpha, alpha-trehalose-phosphate synthase(UDP-	1.67
Bxe_A3572	Putative enoyl-CoA hydratase/isomerase	1.67
Bxe_C0248	Putative enoyl-CoA hydratase/isomerase	1.67
Bxe_A2228	Putative first mannosyl transferase, WbaZ	1.68
Bxe_A2190	Putative aldo/keto oxidoreductase	1.68
Bxe_B2580	Response regulator receiver domain	1.71
Bxe_A1324	Putative myo-inositol catabolism LolE protein	1.72
Bxe_B1895	Putative enoyl-CoA hydratase/isomerase	1.73
Bxe_B1088	Enoyl-CoA hydratase/isomerase family protein	1.73
Bxe_C0251	Electron transfer flavoprotein, alpha subunit,	1.74
Bxe_C1290	Putative tannase and feruloyl esterase	1.75
Bxe_C0249	Putative enoyl-CoA hydratase/isomerase	1.75
Bxe_B0281	Putative short-chain dehydrogenase,	1.75
Bxe_B1818	Putative oxidoreductase	1.76
Bxe_B1400	Betaine-aldehyde dehydrogenase	1.76
Bxe_C0247	Acyl-CoA dehydrogenase	1.77
Bxe_B1085	AMP-binding enzyme	1.77

Bxe_B2862	Putative trehalose synthase	1.77
Bxe_B1579	putative electron transfer flavoprotein beta-	1.77
Bxe_B2864	Putative glycogen operon protein GlgX	1.79
Bxe_A2328	Putative ATP-dependent DNA ligase	1.79
Bxe_B1599	Putative sarcosine oxidase delta subunit	1.79
Bxe_A3588	Cyclohexanone monooxygenase	1.80
Bxe_A3894	Putative D-lactate dehydrogenase	1.80
Bxe_B1401	Ribokinase	1.81
Bxe_A4355	Putative carbon monoxide dehydrogenase, CoxE	1.81
Bxe_A2941	N-formylglutamate deformylase	1.82
Bxe_C0308	Putative short-chain dehydrogenase/reductase	1.84
Bxe_B0460	3-isopropylmalate dehydratase small subunit	1.85
Bxe_A2132	Putative arylsulfatase	1.85
Bxe_C0888	3-hydroxyacyl-CoA dehydrogenase (PaaH)	1.85
Bxe_A0015	Methylmalonate-semialdehyde dehydrogenase	1.86
Bxe_B2300	methylitaconate delta2-delta3-isomerase	1.86
Bxe_A4443	Ethanolamine ammonia-lyase	1.86
Bxe_A3574	Putative acyl-CoA dehydrogenase	1.89
Bxe_C1275	Deoxyribose-phosphate aldolase	1.90
Bxe_A0966	NAD-dependent formate dehydrogenase, gamma	1.91
Bxe_C1274	Betaine-aldehyde dehydrogenase	1.94
Bxe_C1016	Putative tripartite tricarboxylate	1.94
Bxe_A0963	NAD-dependent formate dehydrogenase, delta	1.95
Bxe_A3223	Putative carbonic anhydrase protein	1.95
Bxe_A0964	NAD-dependent formate dehydrogenase, alpha	1.96
Bxe_C1272	Putative ribokinase	1.96
Bxe_B1399	Deoxyribose-phosphate aldolase	1.98
Bxe_A2329	Putative membrane protein, Ku-domain	1.98
Bxe_C1282	Putative ribokinase	1.99
Bxe_B1084	Butyryl-CoA dehydrogenase	2.00
Bxe_C0208	Putative acyl-CoA dehydrogenase	2.04
Bxe_A4441	Aldehyde dehydrogenase (NAD+)	2.07
Bxe_C0083	Putative alcohol dehydrogenase cytochrome c	2.07
Bxe_B2469	Pyrroloquinoline quinone synthesis D	2.08
Bxe_B2863	1,4-alpha-glucan branching enzyme	2.11
Bxe_A2225	UTP--glucose-1-phosphate uridylyltransferase	2.15
Bxe_B0942	Glutamate--ammonia ligase	2.15
Bxe_B1087	3-hydroxyisobutyrate dehydrogenase	2.22
Bxe_B0461	Putative carboxy-phosphonoenolpyruvate mutase	2.22
Bxe_C0204	Alpha-methylacyl-CoA racemase	2.22
Bxe_A1429	Dihydroorotate dehydrogenase 1	2.24
Bxe_A3671	Putative oxidoreductase	2.24
Bxe_B0941	Putative aminotransferase	2.26

Bxe_B0316	Lipoate synthase	2.27
Bxe_C0016	Putative aminotransferase	2.28
Bxe_B1086	Methylmalonate-semialdehyde dehydrogenase	2.29
Bxe_B1089	Enoyl-CoA hydratase/isomerase family	2.30
Bxe_B0314	Acetoin dehydrogenase, beta subunit	2.35
Bxe_B1585	Glycine hydroxymethyltransferase	2.39
Bxe_C0889	Putative enoyl-CoA hydratase/isomerase	2.42
Bxe_B0315	Putative acetoin dehydrogenase	2.46
Bxe_A1427	Amidase, hydantoinase/carbamoylase	2.46
Bxe_C0078	Putative Zinc-binding dehydrogenase	2.49
Bxe_B1582	NADH-flavin oxidoreductase / NADH oxidase family	2.51
Bxe_C0163	NAD+ synthase	2.58
Bxe_B1164	Peptidase C56, PfpI	2.61
Bxe_A1323	Putative myo-inositol catabolism LolB protein	2.61
Bxe_B0313	Pyruvate dehydrogenase (lipoamide)	2.63
Bxe_B0283	Putative fumarate reductase flavoprotein	2.66
Bxe_B0459	3-isopropylmalate dehydratase large subunit	2.82
Bxe_C1023	Putative hydrolase, alpha/beta fold family	2.82
Bxe_B1215	Catalase	2.84
Bxe_A1428	FAD-dependent pyridine nucleotide- disulphide	2.94
Bxe_B0284	Putative MmgE/Prp family protein	3.02
Bxe_C0166	Putative glutathione-dependent formaldehyde	3.15
Bxe_B0458	Putative carboxy-phosphonoenolpyruvate mutase	3.15
Bxe_C0077	Putative glutathione-independent formaldehyde	3.16
Bxe_A2027	Putative acyl-CoA dehydrogenase-related protein	3.34
Bxe_A2028	Putative methyltransferase protein	3.46
Bxe_B0286	Putative MmgE/PrpD family protein	4.12
Bxe_A1431	Putative dihydropyrimidinase	5.44
	Total	**147**
Others		
Bxe_A3737	Putative transposase	-1.64
Bxe_A1097	Putative phage integrase	-1.58
Bxe_A2549	Putative IS66 transposase, TnpB	-1.53
Bxe_C1182	Putative phage integrase	-1.51
Bxe_C1175	Transposition protein TniQ	1.60
Bxe_B0608[c]	Putative autoinducer synthesis protein, BraI	1.64
Bxe_B0319	Phasin	2.28
Bxe_A1459	Bacterioferritin	2.29
	Total	**8**

[a]Negative fold changes represent genes downregulated by the QS system; positive fold changes represent genes upregulated by the QS system.

[b]Hypothetical proteins unique to *B. xenovorans* LB400

[c]Genes previously reported to be controlled by QS.

Table 7.6. BraI/R regulon in *B. phymatum* STM815.

Gene ID	Gene product	Fold change[a]
Hypothetical proteins		
Bphy_0250	Conserved hypothetical protein	-6.37
Bphy_0175	Conserved hypothetical protein	-2.73
Bphy_0234	Conserved hypothetical protein	-2.54
Bphy_1418	Conserved hypothetical protein	-2.48
Bphy_0927	Conserved hypothetical protein	-2.20
Bphy_1217	Hypothetical protein	-2.15
Bphy_0632	Conserved hypothetical protein	-2.13
Bphy_0213	Conserved hypothetical protein	-2.01
Bphy_1428	Conserved hypothetical protein	-1.93
Bphy_1016	Conserved hypothetical protein	-1.91
Bphy_1088	Conserved hypothetical protein	-1.89
Bphy_0026	Conserved hypothetical protein	-1.81
Bphy_0211	Conserved hypothetical protein	-1.77
Bphy_1313	Conserved hypothetical protein	-1.69
Bphy_0814	Conserved hypothetical protein	-1.62
Bphy_0753	Conserved hypothetical protein	-1.60
Bphy_1038	Conserved hypothetical protein	-1.53
Bphy_0812	Conserved hypothetical protein	-1.53
Bphy_0600	Conserved hypothetical protein	1.53
Bphy_0422	Conserved hypothetical protein	1.61
Bphy_1259	Conserved hypothetical protein	1.68
Bphy_1208	Hypothetical protein	1.82
Bphy_0215	Conserved hypothetical protein	1.87
Bphy_1247	Conserved hypothetical protein	1.93
Bphy_1246	Conserved hypothetical protein	1.96
Bphy_1241[b]	Hypothetical protein	2.25
Bphy_0192	Conserved hypothetical protein	2.36
Bphy_1261	Conserved hypothetical protein	2.66
Bphy_1299	Conserved hypothetical protein	4.10
Bphy_0467	Conserved hypothetical protein	4.25
Bphy_1473	Conserved hypothetical protein	9.68
	Total	**31**
Transcriptional regulators		
Bphy_0129	Anti-sigma-factor antagonist	-2.38
Bphy_0352	Transcriptional regulator, LysR family	-2.04
Bphy_1472	Two component LuxR family transcriptional regulator	-2.02
Bphy_0042	Two component Fis family transcriptional regulator	-2.01
Bphy_1008	Transcriptional regulator, MarR family	-1.88
Bphy_0097	Two component LuxR family transcriptional regulator	1.64

Bphy_1272	Transcriptional regulator, TetR family	1.96
Bphy_0645	Two component LuxR family transcriptional regulator	2.67
Bphy_0434	GntR domain-containing protein	2.77
	Total	**9**
DNA/RNA modifications		
Bphy_0867	DNA polymerase III, epsilon subunit	-3.27
Bphy_0868	Ribonuclease H	-3.16
Bphy_0313	Peptidyl-tRNA hydrolase	-3.01
Bphy_0240	Helicase superfamily 1 and 2 ATP-binding	-2.73
Bphy_0242	Integrase family protein	-2.52
Bphy_0527	Putative nucleotide-binding protein	-2.44
Bphy_1358	DNA repair protein RadA	-2.23
Bphy_1448	Single-stranded-DNA-specific exonuclease RecJ	-2.17
Bphy_0465	Sua5/YciO/YrdC/YwlC family protein	-2.06
Bphy_0116	Histone family protein nucleoid-structuring	-1.95
Bphy_0953	Recombination protein RecR	-1.85
Bphy_0738	DNA gyrase, A subunit	-1.82
Bphy_0073	PUA domain-containing protein	-1.81
Bphy_0426	DEAD/DEAH box helicase domain-containing protein	-1.79
Bphy_1444	TatD-related deoxyribonuclease	-1.78
Bphy_0818	Ribonuclease, Rne/Rng family	-1.74
Bphy_0319	Formamidopyrimidine-DNA glycosylase	-1.74
Bphy_1390	Cysteinyl-tRNA synthetase	-1.73
Bphy_1083	ProQ activator of osmoprotectant transporter ProP	-1.69
Bphy_0405	Glycyl-tRNA synthetase, beta subunit	-1.59
Bphy_0875	Transcription elongation factor GreA	1.50
Bphy_0339	Excinuclease ABC, A subunit	1.64
Bphy_0583	Ribosomal protein L33	1.72
Bphy_0584	Ribosomal protein L28	1.88
Bphy_0301	RNA polymerase, sigma 32 subunit, RpoH	1.95
Bphy_0561	Translation elongation factor G	2.32
	Total	**26**
Transporters		
Bphy_1089	Arginine/ornithine antiporter	-3.70
Bphy_0125	Transport-associated	-2.65
Bphy_0647	Efflux transporter, RND family, MFP subunit	-2.73
Bphy_0184	Periplasmic binding protein	-2.50
Bphy_0327	ABC transporter related	-2.45
Bphy_1235	Monosaccharide-transporting ATPase	-2.18
Bphy_0884	Phosphate ABC transporter, inner membrane	-1.87
Bphy_0555	Oligopeptide/dipeptide ABC transporter, ATPase	-1.86
Bphy_0221	Extracellular solute-binding protein	-1.81

Bphy_0206	ABC transporter related	-1.64
Bphy_0797	MATE efflux family protein	1.87
Bphy_0112	Extracellular ligand-binding receptor	1.99
Bphy_1267	RND efflux system, outer membrane lipoprotein	4.78
Bphy_1266	Acriflavin resistance protein	14.13
	Total	**14**
Membrane/ cell wall synthesis and components		
Bphy_1325	Lipid-A-disaccharide synthase	-3.54
Bphy_1417	Outer membrane assembly lipoprotein YfgL	-3.06
Bphy_0154	Porin Gram-negative type	-2.61
Bphy_0448	O-antigen polymerase	-2.50
Bphy_1327	(3R)-hydroxymyristoyl-ACP dehydratase, FabZ	-2.46
Bphy_0454	Lipopolysaccharide heptosyltransferase II	-2.29
Bphy_0403	Phospholipid/glycerol acyltransferase	-2.06
Bphy_0407	Apolipoprotein N-acyltransferase	-1.99
Bphy_0404	Histidinol-phosphate phosphatase family protein	-1.87
Bphy_1288	Basic membrane lipoprotein	-1.79
Bphy_0317	Outer membrane lipoprotein LolB	-1.77
Bphy_0438	Integral membrane sensor signal transduction histidine kinase	-1.76
Bphy_0865	GtrA family protein	-1.68
Bphy_0899	Peptidase M23B	-1.65
Bphy_0573	Lipoprotein signal peptidase	-1.61
Bphy_0396	Organic solvent tolerance protein	-1.50
Bphy_1413	HflK protein	-1.51
Bphy_1451	17 kDa surface antigen	1.64
Bphy_0966	Putative competence lipoprotein, ComI	1.81
Bphy_0293	N-acetylglucosamine-6-phosphate deacetylase	2.43
Bphy_1075	Acyltransferase 3	2.60
	Total	**21**
EPS production		
Bphy_1058	Nucleotide sugar dehydrogenase	-4.10
Bphy_1057	Undecaprenyl-phosphate glucose	-2.90
Bphy_1060	Polysaccharide export protein	-2.51
Bphy_1064	Putative transmembrane protein	-2.26
	Total	**4**
Cellular processes/ Metabolism		
Bphy_0444	Succinyl-CoA synthetase, alpha subunit	-6.39
Bphy_0830	3-oxoacyl-(acyl-carrier-protein) reductase	-3.78
Bphy_0829	Malonyl CoA-acyl carrier protein transacylase	-3.55
Bphy_0846	Beta-N-acetylhexosaminidase	-3.47

Bphy_0287	Electron transport protein SCO1/SenC	-3.39
Bphy_1001	Alkyl hydroperoxide reductase/ Thiol specific	-3.36
Bphy_0845	4'-phosphopantetheinyl transferase, AcpS	-3.26
Bphy_1090	Arginine deiminase	-2.88
Bphy_0844	Pyridoxal phosphate biosynthetic protein PdxJ	-2.62
Bphy_0977	Cystathionine beta-lyase	-2.35
Bphy_1454	Fe-S protein assembly chaperone HscA	-2.26
Bphy_0931	Homoserine dehydrogenase	-2.20
Bphy_0397	SurA domain protein	-2.19
Bphy_0285	Cytochrome oxidase assembly	-2.17
Bphy_0185	Short-chain dehydrogenase/reductase SDR	-2.14
Bphy_0126	Cytochrome c class I	-2.11
Bphy_0398	4-hydroxythreonine-4-phosphate dehydrogenase	-2.07
Bphy_0217	Lipoyl synthase	-2.07
Bphy_0333	Adenine phosphoribosyltransferase	-2.02
Bphy_0186	DSBA oxidoreductase	-2.02
Bphy_1046	Ankyrin	-2.01
Bphy_0394	Nucleotidyl transferase	-2.01
Bphy_0179	Carbonate dehydratase	-2.01
Bphy_0695	Glycosyl transferase family 2	-1.98
Bphy_0994	Pyridoxal-5'-phosphate-dependent protein beta subunit	-1.95
Bphy_0514	Pyridoxamine 5'-phosphate oxidase	-1.91
Bphy_0321	Peptidase S16 lon domain-containing protein	-1.89
Bphy_0162	MaoC domain-containing protein dehydratase	-1.89
Bphy_0571	Luciferase family protein	-1.88
Bphy_0286	Protoheme IX farnesyltransferase	-1.88
Bphy_0395	Aminoglycoside phosphotransferase	-1.78
Bphy_1198	Xylulokinase	-1.73
Bphy_0828	3-oxoacyl-(acyl-carrier-protein) synthase III	-1.72
Bphy_0744	3-phosphoshikimate 1-carboxyvinyltransferase	-1.71
Bphy_1081	4-hydroxyphenylpyruvate dioxygenase	-1.70
Bphy_0974	Ribokinase-like domain-containing protein	-1.69
Bphy_0821	Rieske (2Fe-2S) domain protein	-1.68
Bphy_0745	Cytidylate kinase, cmk	-1.68
Bphy_0811	D-isomer specific 2-hydroxyacid dehydrogenase	-1.68
Bphy_0960	Protein-L-isoaspartate O-methyltransferase	-1.67
Bphy_0935	Molybdopterin biosynthesis MoaE protein	-1.61
Bphy_0620	Riboflavin synthase, alpha subunit	-1.60
Bphy_0713	Xanthine dehydrogenase, small subunit	-1.59
Bphy_0409	ChaC family protein	-1.57
Bphy_0859	Alpha,alpha-trehalose-phosphate synthase	-1.53
Bphy_0371	FAD linked oxidase domain-containing protein	1.52
Bphy_0765	D-amino-acid dehydrogenase	1.53

Bphy_1427	Dihydrodipicolinate synthase	1.55
Bphy_0546	Diguanylate phosphodiesterase	1.55
Bphy_0271	Appr-1-p processing domain protein	1.57
Bphy_0191	Homocysteine S-methyltransferase	1.59
Bphy_1243	Saccharopine dehydrogenase	1.62
Bphy_0710	Adenosine deaminase	1.65
Bphy_0193	Alpha/beta hydrolase fold protein	1.75
Bphy_1294	Hydroxyisourate hydrolase	1.87
Bphy_0711	Xanthine dehydrogenase accessory protein XdhC	1.90
Bphy_0082	Aldo/keto reductase	1.93
Bphy_1475	Methenyltetrahydrofolate cyclohydrolase	1.94
Bphy_0351	Putative periplasmic cytochrome c protein	1.95
Bphy_0027	Thioesterase superfamily protein	2.03
Bphy_0508	Arylformamidase	2.31
Bphy_0507	Kynureninase	2.71
Bphy_0157	2-nitropropane dioxygenase NPD	2.91
Bphy_1078	Phosphoribosyltransferase	3.02
Bphy_1433	Alpha/beta hydrolase fold protein	3.22
Bphy_1029	Nicotinamidase	4.37
Bphy_1028	3-hydroxyacyl-CoA dehydrogenase NAD-binding	5.74
Bphy_0385	Acyl-CoA dehydrogenase domain protein	10.38
	Total	**68**
Others		
Bphy_0656	Septum site-determining protein MinD	-2.39
Bphy_0051	Rod shape-determining protein MreC	-1.95
Bphy_0187	Sporulation domain-containing protein	-1.61
	Total	**3**

[a]Negative fold changes represent genes downregulated by the QS system; positive fold changes represent genes upregulated by the QS system.

[b]Hypothetical proteins unique to *B. xenovorans* LB400

Table 7.7. Number of nodules in plants of *Phaseolus vulgaris* var. Flamingo and var. Negro Jamapa, after inoculation with *B. phymatum* GR01 and mutant derivative GR01BRAI.

P. vulgaris var.	Strain	
	GR01	GR01BRAI
Flamingo	101a	96a
Negro Jamapa	96[a]	97a

Means were separated using the Tukey honestly significant difference (HSD) test ($P \leq 0.05$) with SPSS software. Within a row, numbers followed by the same letter are not significantly different (n = 12). The data shown are representative of two or three independent experiments for Flamingo and Negro Jamapa, respectively.

Table 7.8. Plant dry weight of *Phaseolus vulgaris* var. Negro Jamapa inoculated with *B. phymatum* GR01 and mutant derivatives GR01BRAI

P. vulgaris var.	Strain	
	GR01	GR01BRAI
Negro Jamapa	5.06[a]	4.89a

Means were separated using the Tukey honestly significant difference (HSD) test ($P \leq 0.05$) with SPSS software. Within a row, numbers followed by the same letter are not significantly different (n = 12). The data shown are representative of three independent experiments.

Table 7.9. Primers used in this study.

Primer name	Primer sequence
Arbitrary PCR primers	
Arb1	5'- GGCCACGCGTCGACTAGTACNNNNNNNNNAGCTG -3'
Arb2	5'- GGCCACGCGTCGACTAGTAC -3'
mTn5-GNm_UP_external	5'- TTCTTGTAACGCGCTTTCC -3'
mTn5-GNm_UP_internal	5'- GAATGCCCACAGGCCGTCGAG -3'
mTn5-GNm_DOWN_external	5'- TATTGCTGAAGAGCTTGGC -3'
mTn5-GNm_DOWN_internal	5'- GCATCGCCTTCTATCGCCTTC -3'
***B. kururiensis* M130 16S rRNA primers**	
16S_M130_Fw	5'- TCGGGTTGTAAAGCACTTTTGTCC -3'
16S_M130_RV	5'- CCATCGGTGTTCCTCCACAT -3'

Table 7.10. β-glucuronidase activity of the *B. kururiensis* M130 mTn5-GusNm mutants with statistically significant differences in the levels of this enzyme in the presence and absence of macerated rice plant in liquid medium.

Mutant #	β-glucuronidase activity[1]		*P* value[2]
	Without macerated rice plant	With macerated rice plant	
2	10.63 ±0.99	19.58 ±0.91	0.0003
21	7.32 ±1.08	14.7 ±2.91	0.0148
28	136.48 ±22.97	68.9 ±12.3	0.0109
30	5.14 ±0.94	11.1 ±0.95	0.0015
31	201.91 ±38.56	981.66 ±172.02	0.0016
34	15.16 ±0.58	26.48 ±1.22	0.0001
36	9.33 ±2.01	16.96 ±2.27	0.0121
37	5.88 ±0.68	10.08 ±0.68	0.0016
38	24.49 ±3.23	68.22 ±8.47	0.0011
43	131.27 ±16.49	67.93 ±16.86	0.0097
47	30.65 ±5.44	15.23 ±3.01	0.0129
48	12.16 ±2.34	20.88 ±3.27	0.0198
50	387.54 ±28.76	178.68 ±16.76	0.0004
55	583.64 ±327.88	1445.49 ±167.74	0.0154
57	1326.63 ±86.42	552.52 ±114.91	0.0007
62	23.44 ±3.12	65.19 ±13.93	0.0072
64	163.86 ±15.84	84.34 ±24.09	0.0088
65	256.28 ±45.78	1542.42 ±226.56	0.0006
66	6.49 ±1.18	14.59 ±3.41	0.0177
71	951.17 ±93.02	602.29 ±148.35	0.026
73	82.51 ±5.85	261.9 ±24.59	0.0003
76	11.14 ±1.87	33.62 ±2.23	0.0002
79	7.61 ±1.39	11.29 ±1.46	0.0341
87	29.49 ±5.41	19.85 ±2.33	0.047
99	299.54 ±42.83	31.02 ±5.8	0.0004
103	318.66 ±13.4	154.96 ±20.71	0.0111
121	4.86 ±0.93	8.32 ±1.11	0.0144
124	583.49 ±86.3	27.91 ±4.44	0.0004
123	110.03 ±9.98	408.01 ±13.31	<0.0001
130	4.86 ±1.25	9.24 ±0.28	0.004
131	783 ±243.32	348.72 ±118.47	0.0494
135	4.47 ±0.9	9.09 ±0.72	0.0023
137	918 ±39.52	5697.32 ±1665.41	0.0077
144	2606.45 ±396.16	644.66 ±155.14	0.0013
145	670.2 ±125.56	1287.28 ±176.78	0.0079
146	283.22 ±12.47	1844.51 ±507.89	0.0091
150	1721.59 ±96.32	770.63 ±92.89	0.0003
160	491.86 ±6.87	317.13 ±13.4	<0.0001
163	34.12 ±5.95	83.91 ±6.5	0.0006
164	17.76 ±5.64	67.38 ±3.38	0.0002
178	221.97 ±71.17	84.82 ±42.65	0.0458

187	115.92 ±10.27	56.56 ±3.71	0.0007
193	480.45 ±67.13	1426.02 ±134.09	0.0004
194	3.54 ±0.26	6.92 ±0.43	0.0003
201	365.92 ±26.65	215.36 ±60.91	0.0172
219	5.32 ±1.77	10.25 ±0.99	0.0136
226	1052.59 ±217.11	59.81 ±37.44	0.015
227	81.55 ±2.43	148.52 ±2.69	<0.0001
230	101.37 ±3.3	203.8 ±7.06	<0.0001
243	308.94 ±7.91	145.5 ±39.1	0.0021
244	2402.46 ±200.28	1192.16 ±271.76	0.0034
248	8.01 ±0.3	10.77 ±0.36	0.0005
250	169.59 ±20.47	73.07 ±21.18	0.005
251	7.17 ±0.91	9.83 ±0.48	0.0111
262	7.23 ±0.58	10.63 ±0.77	0.0036
267	226.38 ±72.03	1290.03 ±226.02	0.0015
270	655.95 ±39.73	1472.57 ±62.72	<0.0001
275	29.99 ±1.91	52.9 ±11.91	0.0017

[1] The results presented are mean values ± the standard deviations of three independent biological replicates; [2] Statistical analysis was performed using Student's T-test and the considered cut-off was $P \leq 0.05$.

Table 7.11. Genes of *B. kuruviensis* M130 differentially regulated in the presence of macerated rice plant identified by RNAseq analisys.

Contig	Start	Stop	Strand	Product	Fold change	FDR value
1	3489	3773	+	Phenylacetate-CoA oxygenase2C PaaH subunit	2.75	7.33E-10
1	3782	4585	+	Phenylacetate-CoA oxygenase2C PaaI subunit	2.39	4.95E-03
1	4632	5204	+	Phenylacetate-CoA oxygenase2C PaaJ subunit	2.12	7.82E-03
1	5207	6295	+	Phenylacetate-CoA oxygenase/reductase2C PaaK subunit	2.17	2.90E-06
1	7287	7952	+	Histone acetyltransferase HPA2 and related acetyltransferases	2.08	2.52E-04
1	8796	9893	+	4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27)	4.22	0.00E+00
1	10089	10304	-	putative outer membrane protein	5.42	0.00E+00
1	14479	16770	-	NADP-dependent malic enzyme (EC 1.1.1.40)	-2.15	8.91E-09
1	18700	19386	+	Orotate phosphoribosyltransferase (EC 2.4.2.10)	-2.09	4.12E-36
1	19616	20212	+	Phospholipid-binding protein	6.97	0.00E+00
1	26968	27855	-	Transcriptional regulator2C LysR family	4.36	1.17E-08
1	31074	31841	+	FIG00455878: hypothetical protein	3.30	0.00E+00
1	32477	33388	+	Permease of the drug/metabolite transporter (DMT) superfamily	3.99	4.38E-06
1	35740	36087	+	FIG00461860: hypothetical protein	6.44	1.07E-05
1	36204	36623	-	putative exported protein	4.58	0.00E+00
1	39644	42412	+	PUTATIVE VGR-RELATED PROTEIN	-6.94	3.01E-29
1	42415	43995	+	FIG00459950: hypothetical protein	-7.72	2.38E-14

1	46335	46460	-	hypothetical protein	-7.70	2.35E-04
1	46482	46766	+	hypothetical protein	-2.50	4.36E-03
1	46817	48634	-	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] (EC 2.6.1.16)	-2.60	1.53E-14
1	50261	51319	-	tRNA(Cytosine32)-2-thiocytidine synthetase	-2.27	1.69E-09
1	59321	59989	+	5-formyltetrahydrofolate cyclo-ligase (EC 6.3.3.2)	3.70	2.71E-10
1	60816	61469	+	Allophanate hydrolase 2 subunit 1 (EC 3.5.1.54)	2.45	2.53E-05
1	61466	62515	+	Allophanate hydrolase 2 subunit 2 (EC 3.5.1.54)	2.14	0.00E+00
1	65575	66489	-	Uncharacterized protein conserved in bacteria	-2.03	4.79E-04
1	66729	67697	-	Dipeptide transport ATP-binding protein DppF (TC 3.A.1.5.2)	-6.10	2.75E-30
1	67751	68788	-	Dipeptide transport ATP-binding protein DppD (TC 3.A.1.5.2)	-4.91	5.40E-09
1	69784	70794	-	Dipeptide transport system permease protein DppB (TC 3.A.1.5.2)	-4.40	2.11E-16
1	70926	72557	-	Dipeptide-binding ABC transporter2C periplasmic substrate-binding component (TC 3.A.1.5.2)	-5.28	1.10E-20
1	72841	73671	-	52C10-methylenetetrahydrofolate reductase (EC 1.5.1.20)	-2.24	7.09E-06
1	73687	74040	-	FIG028593: membrane protein	-2.40	8.08E-11
1	78300	79928	-	Choline dehydrogenase (EC 1.1.99.1)	4.08	1.58E-05
1	79929	80087	+	hypothetical protein	3.56	1.88E-05
1	80355	82046	+	Acyl-CoA dehydrogenase (EC 1.3.8.7)	4.48	1.08E-11
1	82208	83626	+	Aldehyde dehydrogenase (EC 1.2.1.3) Probable coniferyl aldehyde dehydrogenase (EC 1.2.1.68)	3.14	2.73E-14
1	87713	89455	+	Flagellar M-ring protein FliF	-2.94	1.39E-04
1	89525	90526	+	Flagellar motor switch protein FliG	2.36	2.61E-13
1	90519	91199	+	Flagellar assembly protein FliH	2.47	2.48E-03
1	100308	100577	-	Flagellar biosynthesis protein FliQ	-7.16	2.45E-04
1	100604	101416	-	Flagellar biosynthesis protein FliP	-3.35	1.88E-17
1	102413	103411	-	Flagellar motor switch protein FliM	-8.70	7.84E-06
1	103490	103990	-	Flagellar biosynthesis protein FliL	-4.66	1.65E-23
1	108977	109909	-	Flagellar protein FlgJ [peptidoglycan hydrolase] (EC 3.2.1.-)	-8.58	4.48E-48
1	109922	111121	-	Flagellar P-ring protein FlgI	-8.37	3.40E-08
1	111862	112650	-	Flagellar basal-body rod protein FlgG	-7.68	1.75E-13
1	112691	113449	-	Flagellar basal-body rod protein FlgF	-10.93	5.13E-68
1	113467	114972	-	Flagellar hook protein FlgE	-9.53	9.98E-98
1	115055	115777	-	Flagellar basal-body rod modification protein FlgD	-5.31	1.26E-05
1	115798	116223	-	Flagellar basal-body rod protein FlgC	-6.40	1.17E-26
1	116442	116936	-	Flagellar basal-body rod protein FlgB	-4.17	1.45E-03
1	119021	119374	+	Negative regulator of flagellin synthesis	2.98	8.49E-04
1	120761	120916	-	hypothetical protein	3.41	1.20E-03
1	121872	122651	-	Flagellar synthesis regulator FleN	-5.76	1.48E-06
1	122761	124860	-	Flagellar biosynthesis protein FlhF	-2.08	8.31E-04
1	125281	127380	-	Flagellar biosynthesis protein FlhA	-3.34	8.43E-08
1	127377	128555	-	Flagellar biosynthesis protein FlhB	-3.30	5.61E-04
1	128566	128949	-	hypothetical protein	-2.32	6.17E-05
1	141092	142126	-	Flagellar motor rotation protein MotB	2.88	2.62E-08
1	142149	143009	-	Flagellar motor rotation protein MotA	3.03	3.91E-03
1	143915	144217	-	Flagellar transcriptional activator FlhD	-3.65	3.20E-10

1	152570	152995	+	conserved hypothetical protein	-2.02	1.40E-20
1	160000	162306	-	TPR domain protein2C putative component of TonB system	-7.82	3.55E-06
1	162371	164128	-	TPR domain protein2C putative component of TonB system	-5.08	1.81E-08
1	164188	164874	-	TPR domain protein	-5.67	3.30E-05
1	165248	166111	+	hypothetical protein	-9.81	3.25E-08
1	166273	166704	+	FIG01210424: hypothetical protein	-7.29	4.84E-09
1	166754	167863	+	Aminotransferase	-10.02	3.12E-10
1	167881	168831	+	hypothetical protein	-3.22	1.50E-04
1	168913	169578	+	transferase2C putative	-6.83	6.47E-03
1	169597	170547	+	FIG01214411: hypothetical protein	-6.86	3.43E-20
1	170563	171501	+	hypothetical protein	-20.36	1.27E-45
1	171531	172352	+	Uncharacterized protein Rv1501/MT1550	-6.70	3.80E-102
1	172349	173443	+	Methyltransferase FkbM family	-5.27	1.35E-17
1	175853	178144	+	Nitric-oxide reductase (EC 1.7.99.7)2C quinol-dependent	9.58	0.00E+00
1	178537	179742	+	Permeases of the major facilitator superfamily	5.67	5.52E-13
1	180312	180719	-	FIG00347697: hypothetical protein	5.56	1.41E-06
1	180971	181441	+	Nitrite-sensitive transcriptional repressor NsrR	70.53	5.31E-05
1	181511	181927	+	PAS/PAC domain (EC 2.7.3.-)	183.49	6.46E-05
1	181992	183194	+	NnrS protein involved in response to NO	24.89	2.07E-08
1	186869	187600	+	thiol:disulfide interchange protein	-2.05	1.22E-03
1	188801	188989	+	hypothetical protein	-4.20	5.22E-11
1	195938	197161	-	DNA-cytosine methyltransferase (EC 2.1.1.37)	-2.15	6.74E-04
1	201265	203463	-	IncI1 plasmid conjugative transfer integral membrane protein TraY	2.41	0.00E+00
1	203480	204001	-	hypothetical protein	2.32	4.02E-06
1	203998	204300	-	hypothetical protein	4.08	2.48E-04
1	204831	206036	-	IncI1 plasmid conjugative transfer protein TraW	3.44	1.66E-09
1	209833	211923	-	ATP-dependent DNA helicase Rep	-2.18	8.33E-09
1	214140	215258	+	Aminomethyltransferase (glycine cleavage system T protein) (EC 2.1.2.10)	-2.72	2.73E-23
1	215337	215717	+	Glycine cleavage system H protein	-4.01	1.14E-28
1	215846	218797	+	Glycine dehydrogenase [decarboxylating] (glycine cleavage system P protein) (EC 1.4.4.2)	-3.21	6.11E-04
1	218824	219018	-	hypothetical protein	-2.96	1.17E-04
1	218977	220107	+	FIG00453951: hypothetical protein	-2.25	7.17E-20
1	220154	221542	+	L-serine dehydratase (EC 4.3.1.17)	-3.41	6.00E-38
1	224875	228825	-	Transcriptional repressor of PutA and PutP / Proline dehydrogenase (EC 1.5.99.8) (Proline oxidase) / Delta-1-pyrroline-5-carboxylate dehydrogenase (EC 1.5.1.12)	4.17	0.00E+00
1	235187	236932	-	Acetoacetyl-CoA synthetase (EC 6.2.1.16) / Long-chain-fatty-acid--CoA ligase (EC 6.2.1.3)	-2.14	1.80E-42
1	237176	237601	-	ATP synthase epsilon chain (EC 3.6.3.14)	-6.64	3.06E-42
1	237774	239168	-	ATP synthase beta chain (EC 3.6.3.14)	-6.76	4.50E-158
1	239275	240159	-	ATP synthase gamma chain (EC 3.6.3.14)	-6.01	5.95E-70
1	240254	241795	-	ATP synthase alpha chain (EC 3.6.3.14)	-3.77	3.66E-07
1	241844	242383	-	ATP synthase delta chain (EC 3.6.3.14)	-2.87	7.26E-03
1	242386	242856	-	ATP synthase F0 sector subunit b	-2.70	9.25E-30
1	242866	242982	-	hypothetical protein	-2.90	1.93E-34
1	242997	243266	-	ATP synthase F0 sector subunit c	-2.55	1.45E-08

1	243344	244195	-	ATP synthase F0 sector subunit a	-2.02	5.72E-12
1	258080	258223	+	hypothetical protein	3.28	1.96E-04
1	260480	261697	-	Leucine-2C isoleucine-2C valine-2C threonine-2C and alanine-binding protein	-2.68	3.09E-26
1	269402	270949	+	Signal transduction histidine kinase	-2.69	1.37E-13
1	280913	281191	+	DNA-binding protein HU-beta	-2.92	1.56E-80
1	283765	286212	+	General secretion pathway protein D	-2.06	1.44E-13
1	286209	287759	+	General secretion pathway protein E	-2.33	4.13E-26
1	287766	288983	+	General secretion pathway protein F	-2.20	4.73E-07
1	289000	289410	-	General secretion pathway protein C	-3.60	3.23E-06
1	300248	300757	-	FIG00457549: hypothetical protein	5.19	0.00E+00
1	303896	305080	-	ADA regulatory protein / Methylated-DNA--protein-cysteine methyltransferase (EC 2.1.1.63)	2.40	3.65E-03
1	306056	306613	-	YaeQ protein	2.11	5.08E-03
1	306588	306701	+	hypothetical protein	2.03	8.53E-06
1	307045	307386	-	FIG00457576: hypothetical protein	6.17	0.00E+00
1	308941	309108	+	hypothetical protein	3.34	5.63E-06
1	309158	309334	+	hypothetical protein	6.09	9.44E-15
1	311000	312280	-	hypothetical protein	2.26	0.00E+00
1	319236	319535	-	miscellaneous%3B unknown	5.00	4.46E-08
1	324657	326315	-	Inner membrane protein translocase component YidC2C long form	-2.63	5.49E-03
1	327734	328051	-	hypothetical protein	2.81	1.54E-11
1	335272	335769	-	FIG004694: Hypothetical protein	3.53	0.00E+00
1	335772	336623	-	Beta-propeller domains of methanol dehydrogenase type	8.69	0.00E+00
1	336620	337246	-	LemA family protein	46.68	0.00E+00
1	344480	345883	+	Ethanolamine permease	2.38	3.37E-04
1	347351	348145	+	Ethanolamine ammonia-lyase light chain (EC 4.3.1.7)	4.19	5.62E-04
1	348204	348596	-	Uncharacterized protein conserved in bacteria	9.40	2.59E-13
1	348815	350335	-	Aldehyde dehydrogenase (EC 1.2.1.3)	12.03	2.48E-15
1	350327	350575	+	hypothetical protein	12.60	0.00E+00
1	356203	357150	+	FIG022886: Transcriptional regulator2C LysR family	4.73	3.69E-12
1	363086	364945	+	Phosphoenolpyruvate carboxykinase [GTP] (EC 4.1.1.32)	2.22	1.30E-11
1	365296	366393	+	Transcriptional regulator2C LysR family	3.09	2.93E-06
1	369460	369837	-	Thiol-disulfide isomerase and thioredoxins	8.47	2.48E-15
1	369918	371174	-	Rossmann fold nucleotide-binding protein Smf possibly involved in DNA uptake	28.24	2.55E-09
1	371416	371964	+	Peptide deformylase (EC 3.5.1.88)	3.69	8.91E-05
1	372035	373045	+	Methionyl-tRNA formyltransferase (EC 2.1.2.9)	2.28	2.31E-07
1	384470	385474	-	Rod shape-determining protein RodA	-4.57	8.04E-19
1	385444	388062	-	Penicillin-binding protein 2 (PBP-2)	-2.79	9.11E-06
1	394908	395747	+	UDP-galactose-lipid carrier transferase (EC 2.-.-.-)	-2.34	3.87E-11
1	395814	396599	+	Exodeoxyribonuclease III (EC 3.1.11.2)	-2.94	5.45E-40
1	399220	399828	-	Homoserine O-acetyltransferase (EC 2.3.1.31)	-2.32	6.16E-07
1	404424	405767	+	Sensor histidine kinase PrrB (RegB) (EC 2.7.3.-)	5.13	1.11E-06
1	405928	406470	+	Dna binding response regulator PrrA (RegA)	6.56	1.06E-03
1	406569	407918	-	ATP-dependent hsl protease ATP-binding subunit HslU	3.61	0.00E+00

1	407953	408489	-	ATP-dependent protease HslV (EC 3.4.25.-)	6.97	1.99E-08
1	417847	418224	-	FIG00454842: hypothetical protein	4.52	1.63E-06
1	420027	421070	+	Oxidoreductase2C aldo/keto reductase family	-2.03	2.86E-06
1	421330	422454	+	hypothetical protein	6.57	0.00E+00
1	422617	422802	-	FIG00452440: hypothetical protein	13.32	7.84E-12
1	436399	437286	-	carbon monoxide dehydrogenase D protein	-2.20	3.42E-03
1	447182	448012	-	Endonuclease/Exonuclease/phosphatase family protein	2.80	0.00E+00
1	448009	448497	-	FIG00452923: hypothetical protein	3.97	1.03E-13
1	450051	450308	-	hypothetical protein	2.07	2.93E-03
1	450288	451439	+	Aminobutyraldehyde dehydrogenase (EC 1.2.1.19)	2.01	9.44E-15
1	456509	457654	+	Leucine-2C isoleucine-2C valine-2C threonine-2C and alanine-binding protein	-2.33	3.85E-31
1	458961	460004	-	PE_PGRS family protein	3.79	2.62E-08
1	463762	465090	+	Cobalt-zinc-cadmium resistance protein CzcD	3.09	0.00E+00
1	469545	469979	+	Predicted endonuclease distantly related to archaeal Holliday junction resolvase	2.17	1.17E-13
1	480955	481341	+	hypothetical protein	3.19	0.00E+00
1	481718	483367	+	hypothetical protein	4.26	3.29E-12
1	485575	486840	+	hypothetical protein	6.70	2.19E-05
1	488794	490278	-	hypothetical protein	2.34	0.00E+00
1	493783	495768	-	Cytochrome C553 (soluble cytochrome f)	2.02	5.14E-05
1	496969	497259	-	FIG146805: Plasmid related protein	2.49	3.40E-10
1	499073	499990	-	FIG00460733: hypothetical protein	2.30	3.21E-04
1	500056	500997	-	Phage-related protein	2.91	1.94E-03
1	515019	517052	+	conserved hypothetical protein VrlP	2.57	0.00E+00
1	518243	519250	-	Fructose repressor FruR2C LacI family	2.47	1.87E-03
1	521995	523083	+	1-phosphofructokinase (EC 2.7.1.56)	3.32	9.82E-03
1	523080	524936	+	PTS system2C fructose-specific IIB component (EC 2.7.1.69) / PTS system2C fructose-specific IIC component (EC 2.7.1.69)	2.26	9.37E-04
1	534773	538396	-	Indolepyruvate ferredoxin oxidoreductase2C alpha and beta subunits	-2.83	3.15E-36
1	539899	540144	-	FIG00454702: hypothetical protein	8.15	0.00E+00
1	540314	540535	-	FIG00453681: hypothetical protein	2.54	1.09E-04
1	540676	541659	-	Fructokinase (EC 2.7.1.4)	2.55	2.56E-05
1	541656	543308	-	D-mannose isomerase (EC 5.3.1.7)	2.67	2.96E-14
1	543305	544294	-	Transcriptional regulators	2.98	5.15E-14
1	547105	548166	-	Sodium/bile acid symporter family	5.88	0.00E+00
1	548154	548270	+	hypothetical protein	5.49	1.35E-04
1	548534	548659	-	hypothetical protein	4.37	3.14E-04
1	548660	549151	+	FIG00452481: hypothetical protein	3.73	4.12E-11
1	558036	558569	-	Leucine-responsive regulatory protein2C regulator for leucine (or lrp) regulon and high-affinity branched-chain amino acid transport system	2.10	3.72E-04
1	560797	561261	-	Acyl dehydratase	-2.78	7.47E-08
1	562063	562989	-	Hydroxymethylglutaryl-CoA lyase (EC 4.1.3.4)	-2.70	6.43E-06
1	564253	564891	+	Predicted esterase of the alpha/beta hydrolase fold	-23.44	8.39E-51
1	564931	566004	-	Biotin synthase (EC 2.8.1.6)	-32.33	1.76E-03
1	566045	566812	-	Dethiobiotin synthetase (EC 6.3.3.3)	-14.22	4.18E-274
1	566809	567999	-	8-amino-7-oxononanoate synthase (EC 2.3.1.47)	-9.46	2.71E-83

1	568013	569353	-	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase (EC 2.6.1.62)	-7.95	2.10E-114
1	572027	572347	-	UPF0225 protein YchJ	-5.30	1.37E-05
1	572511	573641	-	Butyryl-CoA dehydrogenase (EC 1.3.99.2)	-5.02	2.03E-55
1	573713	574390	-	Short-chain dehydrogenase/reductase SDR	-3.19	3.40E-05
1	575624	576394	-	Carbonic anhydrase (EC 4.2.1.1)	-2.40	2.80E-08
1	580648	582429	-	Dipeptide-binding ABC transporter2C periplasmic substrate-binding component (TC 3.A.1.5.2)	-2.32	2.07E-37
1	593061	593318	-	FIG00452673: hypothetical protein	6.22	1.75E-10
1	593313	593432	+	hypothetical protein	3.81	0.00E+00
1	612506	612832	+	FIG00453134: hypothetical protein	7.10	5.69E-04
1	612889	613665	+	Octanoate-[acyl-carrier-protein]-protein-N-octanoyltransferase	2.12	1.07E-05
1	624594	626030	+	Ammonium transporter	4.04	5.06E-13
1	630043	631785	+	Phosphoenolpyruvate-protein phosphotransferase of PTS system (EC 2.7.3.9)	2.60	0.00E+00
1	640575	641051	+	Probable transmembrane protein	-2.39	1.50E-03
1	641175	642713	+	Cytochrome c oxidase polypeptide II (EC 1.9.3.1)	-8.20	8.51E-65
1	642697	642813	-	hypothetical protein	-15.54	5.47E-35
1	642799	644370	+	Cytochrome c oxidase polypeptide I (EC 1.9.3.1)	-8.38	1.94E-120
1	644476	644610	+	hypothetical protein	-6.50	3.49E-07
1	644642	645283	+	Cytochrome oxidase biogenesis protein Cox11-CtaG2C copper delivery to Cox1	-8.08	1.87E-63
1	645319	645546	+	Probable transmembrane protein	-6.98	1.30E-24
1	645689	646546	+	Cytochrome c oxidase polypeptide III (EC 1.9.3.1)	-4.13	3.26E-66
1	646984	647709	+	Cytochrome oxidase biogenesis protein Surf12C facilitates heme A insertion	-3.30	4.52E-10
1	647927	648481	+	Probable transmembrane protein	-4.35	2.45E-81
1	657598	658332	+	Predicted transcriptional regulator of N-Acetylglucosamine utilization2C GntR family	5.61	8.49E-08
1	658434	659537	+	N-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25)	2.71	2.86E-04
1	659521	660546	+	Glucosamine-6-phosphate deaminase [isomerizing]2C alternative (EC 3.5.99.6)	4.48	6.06E-06
1	660559	663201	+	Phosphoenolpyruvate-protein phosphotransferase of PTS system (EC 2.7.3.9) / PTS system2C glucose-specific IIA component (EC 2.7.1.69)	2.54	3.72E-06
1	663340	665121	+	PTS system2C N-acetylglucosamine-specific IIA component (EC 2.7.1.69) / PTS system2C N-acetylglucosamine-specific IIB component (EC 2.7.1.69) / PTS system2C N-acetylglucosamine-specific IIC component (EC 2.7.1.69)	2.14	6.68E-14
1	665254	665451	-	hypothetical protein	16.71	0.00E+00
1	665462	666598	-	Cytochrome d ubiquinol oxidase subunit II (EC 1.10.3.-)	30.69	0.00E+00
1	666617	668206	-	Cytochrome d ubiquinol oxidase subunit I (EC 1.10.3.-)	41.90	0.00E+00
1	668570	670417	-	Transport ATP-binding protein CydC	2.60	3.35E-08
1	670422	672215	-	Transport ATP-binding protein CydD	2.62	0.00E+00
1	672546	673481	-	RNA polymerase sigma factor RpoH	6.71	0.00E+00
1	673839	674402	+	Probable signal peptide protein	2.85	3.22E-06
1	674452	674835	+	FIG00453537: hypothetical protein	2.25	8.35E-04
1	676522	676653	+	hypothetical protein	2.03	2.22E-04
1	677987	678127	-	hypothetical protein	2.53	6.22E-03
1	680844	681053	-	hypothetical protein	4.69	8.16E-03
1	684825	685445	-	LSU ribosomal protein L25p	-2.65	2.20E-10
1	692955	693848	-	Hypothetical ATP-binding protein UPF00422C contains P-loop	2.44	1.37E-07

1	693915	694883	-	HPr kinase/phosphorylase (EC 2.7.1.-) (EC 2.7.4.-)	2.75	7.93E-06
1	695830	696189	-	Ribosome hibernation protein YhbH	2.20	7.91E-13
1	704744	705358	+	L-lysine permease	-2.81	9.94E-07
1	706322	707191	+	Formyltetrahydrofolate deformylase (EC 3.5.1.10)	-2.79	1.85E-11
1	714919	715893	+	Threonine dehydratase2C catabolic (EC 4.3.1.19)	-2.23	4.48E-08
1	720321	721214	-	STAPHYLOLYTIC protease PREPROENZYME LASA	2.49	6.56E-05
1	727539	727928	+	Bona fide RidA/YjgF/TdcF/RutC subgroup	-2.11	9.05E-03
1	743012	744814	+	Aspartyl-tRNA synthetase (EC 6.1.1.12) @ Aspartyl-tRNA(Asn) synthetase (EC 6.1.1.23)	-2.28	2.65E-45
1	747037	747636	+	Predicted transcriptional regulator for fatty acid degradation FadP2C TetR family	6.05	1.86E-05
1	747711	749498	+	Acyl-CoA dehydrogenase (EC 1.3.8.7)	8.00	8.74E-12
1	749631	752069	+	Enoyl-CoA hydratase (EC 4.2.1.17) / 32C2-trans-enoyl-CoA isomerase (EC 5.3.3.8) / 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35)	7.37	2.48E-15
1	752100	753299	+	3-ketoacyl-CoA thiolase (EC 2.3.1.16) @ Acetyl-CoA acetyltransferase (EC 2.3.1.9)	4.10	7.58E-03
1	755895	759011	+	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)	-2.03	2.32E-04
1	763640	766012	+	Outer membrane protein Imp2C required for envelope biogenesis / Organic solvent tolerance protein precursor	-2.17	1.59E-70
1	766109	767476	+	Survival protein SurA precursor (Peptidyl-prolyl cis-trans isomerase SurA) (EC 5.2.1.8)	-2.62	1.08E-07
1	767506	768531	+	4-hydroxythreonine-4-phosphate dehydrogenase (EC 1.1.1.262)	-2.12	2.43E-27
1	768584	769429	+	SSU rRNA (adenine(1518)-N(6)/adenine(1519)-N(6))-dimethyltransferase (EC 2.1.1.182)	-3.42	2.28E-05
1	769398	770357	-	Permease of the drug/metabolite transporter (DMT) superfamily	-3.04	6.40E-76
1	773257	773643	+	Lactoylglutathione lyase (EC 4.4.1.5)	-3.03	1.29E-10
1	773645	774577	-	Putative predicted metal-dependent hydrolase	-2.02	2.98E-09
1	778186	779193	-	Glycyl-tRNA synthetase alpha chain (EC 6.1.1.14)	-2.07	1.88E-48
1	788317	789033	+	Predicted transcriptional regulators	2.68	1.28E-03
1	789213	789488	-	hypothetical protein	2.61	2.55E-09
1	791273	791980	-	Glycerol uptake facilitator protein	-3.13	3.13E-13
1	792112	793611	-	Glycerol kinase (EC 2.7.1.30)	-2.42	7.48E-11
1	795560	795949	+	FIG00453008: hypothetical protein	3.32	3.13E-05
1	796210	796407	-	FIG00453722: hypothetical protein	2.49	1.94E-03
1	797775	799412	+	Gamma-glutamyltranspeptidase (EC 2.3.2.2)	4.27	2.21E-11
1	799695	800528	+	FIG00453399: hypothetical protein	3.22	0.00E+00
1	800587	801105	+	FIG00453616: hypothetical protein	3.13	8.01E-06
1	803919	805214	-	Putative diheme cytochrome c-553	-3.81	3.19E-154
1	805236	805958	-	Probable cytochrome c2	-2.15	3.32E-22
1	807679	807981	-	FIG00456601: hypothetical protein	-4.46	7.48E-03
1	820479	821873	-	protein C	3.02	9.44E-15
1	832836	833426	-	hypothetical protein	5.64	2.53E-03
1	839473	841623	+	IncW plasmid conjugative protein TrwB (TraD homolog)	3.72	1.09E-03
1	841804	842181	-	hypothetical protein	3.52	3.71E-07
1	849813	850265	-	hypothetical protein	2.03	3.04E-09
1	850406	851644	-	Integrase	3.27	0.00E+00
1	858165	858536	+	Regulatory protein RecX	2.40	2.30E-09
1	869829	870275	-	Alternative dihydrofolate reductase 3	2.04	4.43E-06
1	879194	880084	+	Phosphoribosylaminoimidazole-succinocarboxamide synthase	-2.03	1.73E-12

				(EC 6.3.2.6)		
1	880117	880656	+	Phosphoribosylaminoimidazole carboxylase catalytic subunit (EC 4.1.1.21)	-2.48	6.03E-31
1	880717	881928	+	Phosphoribosylaminoimidazole carboxylase ATPase subunit (EC 4.1.1.21)	-2.08	7.83E-20
1	881943	883004	+	TsaC protein (YrdC domain) required for threonylcarbamoyladenosine t(6)A37 modification in tRNA	-2.01	7.62E-06
1	889858	891360	+	HtrA protease/chaperone protein	-2.03	4.48E-07
1	892119	892232	-	hypothetical protein	2.30	1.10E-12
1	892317	892676	+	Uncharacterized protein conserved in bacteria	3.38	2.10E-05
1	892722	893459	+	carbon monoxide dehydrogenase G protein	4.59	9.82E-08
1	893569	894204	+	Amidases related to nicotinamidase	8.44	3.60E-03
1	894258	894923	-	Transcription repressor of multidrug efflux pump acrAB operon2C TetR (AcrR) family	11.36	0.00E+00
1	896661	899873	+	RND efflux system2C inner membrane transporter CmeB	-2.61	1.00E-04
1	911073	911855	+	Sorbitol dehydrogenase (EC 1.1.1.14)	4.60	0.00E+00
1	919935	921356	-	D-arabinitol 4-dehydrogenase (EC 1.1.1.11)	6.49	0.00E+00
1	926627	927715	+	Sorbitol dehydrogenase (EC 1.1.1.14)	2.96	8.86E-04
1	927734	929278	+	Xylulose kinase (EC 2.7.1.17)	4.07	0.00E+00
1	936153	937091	-	Transcriptional regulators2C LysR family	4.30	3.45E-08
1	937877	938452	+	Probable signal peptide protein	-2.06	2.08E-04
1	938504	939079	+	Protein yceI precursor	-2.06	8.82E-03
1	940182	941402	+	Serine--glyoxylate aminotransferase (EC 2.6.1.45)	15.97	6.93E-05
1	941413	942225	+	2-keto-4-pentenoate hydratase (EC 4.2.1.80)	28.94	0.00E+00
1	942222	945311	+	Glycolate dehydrogenase (EC 1.1.99.14)2C subunit GlcD	2.90	8.85E-06
1	951159	951989	+	hypothetical protein	-2.77	4.55E-08
1	951986	953149	+	Glycosyltransferase	-3.20	1.45E-03
1	957418	957561	-	hypothetical protein	-2.38	8.85E-06
1	957565	958626	+	dTDP-glucose 42C6-dehydratase (EC 4.2.1.46)	-3.30	1.32E-03
1	958630	959529	+	Glucose-1-phosphate thymidylyltransferase (EC 2.7.7.24)	-3.85	6.31E-06
1	959526	960074	+	dTDP-4-dehydrorhamnose 32C5-epimerase (EC 5.1.3.13)	-3.78	1.05E-03
1	960141	961370	+	Polysaccharide export lipoprotein Wza	-8.59	5.44E-07
1	961367	961831	+	Low molecular weight protein tyrosine phosphatase (EC 3.1.3.48)	-5.20	5.23E-04
1	961839	964106	+	Tyrosine-protein kinase Wzc (EC 2.7.10.2)	-6.24	3.12E-26
1	964986	966356	-	UDP-glucose dehydrogenase (EC 1.1.1.22)	-3.62	9.39E-31
1	966572	967849	-	hypothetical protein	-2.76	8.85E-03
1	968038	970176	-	hypothetical protein	-2.05	1.68E-03
1	983097	983483	-	Probable transmembrane protein	2.06	1.68E-11
1	983598	983873	-	Probable transmembrane protein	2.90	1.82E-03
1	988432	989817	+	4-hydroxybenzoate transporter	5.67	0.00E+00
1	989892	991220	+	Homogentisate 12C2-dioxygenase (EC 1.13.11.5)	8.79	3.69E-12
1	991217	992584	+	Fumarylacetoacetase (EC 3.7.1.2)	4.97	2.52E-06
1	998253	999536	+	Serine--pyruvate aminotransferase (EC 2.6.1.51)	2.13	3.45E-09
1	1001105	1002646	+	Xanthine dehydrogenase2C iron-sulfur cluster and FAD-binding subunit A (1.17.1.4)	9.91	9.95E-04
1	1002743	1005151	+	Xanthine dehydrogenase2C molybdenum binding subunit (EC 1.17.1.4)	8.06	1.54E-03
1	1005244	1005789	+	XdhC protein (assists in molybdopterin insertion into xanthine dehydrogenase)	9.76	9.95E-03

1	1005743	1006753	-	Transcriptional regulator2C LysR family	3.29	2.10E-08
1	1010146	1010505	+	Predicted periplasmic or secreted lipoprotein	3.21	1.46E-05
1	1012948	1013466	-	hypothetical protein	5.73	0.00E+00
1	1013494	1015611	+	D(-)-3-hydroxybutyrate oligomer hydrolase (EC 3.1.1.22)	4.77	1.20E-07
1	1021528	1021890	+	Permeases of the major facilitator superfamily	-5.96	2.43E-06
1	1022102	1022539	+	Copper resistance protein CopC	-13.99	7.06E-04
1	1022686	1023201	+	OpgC	-65.99	1.14E-15
1	1023341	1023913	+	Conserved membrane protein in copper uptake2C YcnI	-40.58	3.86E-49
1	1023985	1026093	+	Outer membrane receptor proteins2C mostly Fe transport	-31.41	1.37E-95
1	1026163	1026651	+	Copper metallochaperone2C bacterial analog of Cox17 protein	-11.10	3.39E-03
1	1030753	1033506	+	Penicillin acylase II	2.57	1.61E-03
1	1052994	1053497	+	Ni2CFe-hydrogenase I cytochrome b subunit	7.99	2.06E-05
1	1053463	1054245	+	Ni2CFe-hydrogenase I cytochrome b subunit	4.09	2.93E-03
1	1054290	1054970	+	Two-component system response regulator QseB	41.92	7.10E-06
1	1054967	1056352	+	Sensory histidine kinase QseC	3.65	4.83E-03
1	1056523	1056999	+	hypothetical protein	28.80	0.00E+00
1	1057109	1058065	-	Cobalt-zinc-cadmium resistance protein	16.19	1.15E-10
1	1058143	1058826	-	Probable transmembrane protein	12.64	0.00E+00
1	1062371	1062994	+	Ni2CFe-hydrogenase I cytochrome b subunit	5.05	9.71E-06
1	1063693	1065042	+	Signal transduction histidine kinase	29.66	7.10E-14
1	1065107	1066057	-	Universal stress protein family	12.48	3.85E-14
1	1066484	1066867	-	Probable transmembrane protein	10.15	3.29E-09
1	1067589	1068170	+	Hemerythrin HHE cation binding region	103.24	0.00E+00
1	1070296	1072035	+	Quino(hemo)protein alcohol dehydrogenase2C PQQ-dependent (EC 1.1.99.8)	8.11	1.46E-05
1	1073671	1074867	-	Coenzyme PQQ synthesis protein E	8.42	2.10E-09
1	1074872	1075153	-	Coenzyme PQQ synthesis protein D	28.16	0.00E+00
1	1075150	1075899	-	Pyrroloquinoline-quinone synthase (EC 1.3.3.11)	16.42	0.00E+00
1	1075902	1076825	-	Coenzyme PQQ synthesis protein B	28.46	3.38E-05
1	1077392	1079470	-	Transcriptional activator of acetoin dehydrogenase operon AcoR	4.72	9.84E-11
1	1082886	1084457	+	hypothetical protein	2.56	8.40E-04
1	1085763	1087160	+	Chloride channel protein EriC	5.43	1.40E-13
1	1087287	1087958	-	Transcriptional regulator2C LysR family	7.40	0.00E+00
1	1088435	1089076	+	hypothetical protein	2.58	2.12E-06
1	1089340	1090089	-	Putative DNA-binding protein in cluster with Type I restriction-modification system	3.56	1.84E-14
1	1090445	1091044	+	short-chain dehydrogenase/reductase SDR	3.11	1.24E-03
1	1091284	1092765	-	Permeases of the major facilitator superfamily	3.73	1.79E-06
1	1092815	1093411	-	Transcriptional regulator2C TetR family	4.67	1.62E-14
1	1094283	1095074	+	Lipid A biosynthesis lauroyl acyltransferase (EC 2.3.1.-)	2.68	2.31E-10
1	1095582	1096313	-	FIG00771309: hypothetical protein	3.23	0.00E+00
1	1096393	1096545	-	hypothetical protein	3.86	1.18E-05
1	1098365	1098577	-	SSU ribosomal protein S21p	9.98	4.52E-11
1	1098772	1098909	-	hypothetical protein	16.82	1.05E-10
1	1099273	1099476	-	Cold shock protein CspG	3.75	0.00E+00
1	1100446	1100712	-	hypothetical protein	6.53	4.21E-07

1	1101834	1102118	+	hypothetical protein	10.49	7.45E-13
1	1104135	1104425	+	Heat shock protein 60 family co-chaperone GroES	9.23	7.76E-09
1	1104471	1106114	+	Heat shock protein 60 family chaperone GroEL	8.06	0.00E+00
1	1106383	1106919	+	Adenylylsulfate kinase (EC 2.7.1.25)	-2.10	1.28E-49
1	1110893	1111327	+	Putative Holliday junction resolvase YqgF	2.08	0.00E+00
1	1125527	1127053	+	hypothetical protein	-3.11	6.30E-07
1	1127050	1128171	+	UDP-N-acetylglucosamine 2-epimerase (EC 5.1.3.14)	-3.74	2.17E-22
1	1128164	1129408	+	Glycosyltransferase	-3.01	2.10E-05
1	1129421	1130392	+	glycosyl transferase group 1	-3.95	8.49E-20
1	1130463	1131614	+	3-dehydroquinate synthase (EC 4.2.3.4)	-3.75	5.48E-06
1	1131883	1132977	+	Mannosyltransferase	-4.91	2.77E-17
1	1132956	1133708	+	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)	-3.35	2.57E-54
1	1133872	1135617	+	glycosyl transferase2C group 1	-7.30	1.47E-63
1	1135605	1138169	+	glycosyl transferase2C group 1 family protein	-5.93	2.79E-38
1	1138258	1139289	+	GDP-mannose 42C6-dehydratase (EC 4.2.1.47)	-7.90	1.33E-160
1	1139350	1140504	+	Glycosyltransferase	-7.79	5.08E-15
1	1140494	1141984	+	Mannosyltransferase	-4.69	1.38E-92
1	1142924	1143076	-	hypothetical protein	-7.68	2.60E-76
1	1143776	1145629	-	Phosphoglycerol transferase I (EC 2.7.8.20)	-4.61	2.95E-25
1	1145684	1146643	-	Glycosyltransferase	-4.76	5.16E-30
1	1146640	1147008	-	hypothetical protein	-3.84	4.73E-05
1	1147067	1147996	-	Transketolase2C C-terminal section (EC 2.2.1.1)	-2.95	8.05E-20
1	1147993	1148799	-	Transketolase2C N-terminal section (EC 2.2.1.1)	-2.08	4.03E-22
1	1154628	1155659	+	Undecaprenyl-phosphate N-acetylglucosaminyl 1-phosphate transferase (EC 2.7.8.-)	6.64	0.00E+00
1	1155660	1157546	+	Nucleoside-diphosphate sugar epimerase/dehydratase	3.61	0.00E+00
1	1157595	1158701	-	Undecaprenyl-phosphate N-acetylglucosaminyl 1-phosphate transferase (EC 2.7.8.-)	3.29	0.00E+00
1	1164892	1166553	+	3-methylmercaptopropionyl-CoA ligase (DmdB)	2.13	2.45E-07
1	1166732	1167349	+	hypothetical protein	7.20	3.71E-05
1	1193085	1193426	-	hypothetical protein	-4.51	2.31E-20
1	1193485	1194045	-	3-hydroxydecanoyl-[ACP] dehydratase (EC 4.2.1.60)	-4.70	1.11E-16
1	1194861	1196114	-	3-oxoacyl-[ACP] synthase (EC 2.3.1.41) FabV like	-4.94	6.26E-08
1	1197114	1199714	-	FIG021862: membrane protein2C exporter	-3.76	3.48E-08
1	1200449	1201435	-	Lysophospholipid acyltransferase	-4.33	2.67E-07
1	1201432	1203255	-	FIGfam138462: Acyl-CoA synthetase2C AMP-(fatty) acid ligase / (3R)-hydroxymyristoyl-[ACP] dehydratase (EC 4.2.1.-)	-3.50	1.28E-17
1	1203427	1204104	-	FIG017861: hypothetical protein	-2.02	3.04E-03
1	1206828	1207862	-	UDP-glucose 4-epimerase (EC 5.1.3.2)	-3.48	3.26E-05
1	1226183	1227394	-	Urea ABC transporter2C permease protein UrtC	2.78	1.35E-05
1	1229149	1230447	-	Urea ABC transporter2C urea binding protein	4.71	1.04E-03
1	1232432	1233532	+	Transcriptional regulator2C LacI family	5.21	2.38E-08
1	1244335	1244886	+	Oxidoreductase probably involved in sulfite reduction	-2.54	2.98E-07
1	1246723	1248042	+	Sulfate adenylyltransferase subunit 1 (EC 2.7.7.4)	-2.92	6.48E-12
1	1249396	1250544	-	FIG000906: Predicted Permease	-2.96	7.96E-23
1	1253875	1254420	+	FIG00452715: hypothetical protein	-2.22	2.80E-06
1	1256809	1257828	+	Transcriptional regulator	2.30	0.00E+00

1	1262275	1264263	+	Outer membrane vitamin B12 receptor BtuB	-6.56	5.40E-108
1	1265307	1266119	+	Iron(III) dicitrate transport ATP-binding protein fecE	-4.39	2.03E-03
1	1266324	1267379	+	Nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase (EC 2.4.2.21)	-5.08	3.99E-26
1	1268302	1268946	+	Alpha-ribazole-5'-phosphate phosphatase (EC 3.1.3.73)	-2.92	1.14E-04
1	1269010	1269984	-	Vitamin B12 ABC transporter2C B12-binding component BtuF	-4.22	1.09E-05
1	1276401	1276787	-	Aspartate 1-decarboxylase (EC 4.1.1.11)	-2.08	3.82E-05
1	1292043	1294322	+	Arginine decarboxylase (EC 4.1.1.19)%3B Ornithine decarboxylase (EC 4.1.1.17)%3B Lysine decarboxylase (EC 4.1.1.18)	-2.24	1.06E-57
1	1295282	1296826	-	Argininosuccinate lyase (EC 4.3.2.1)	-2.45	4.38E-35
1	1305426	1306616	+	Uncharacterized protein EC-HemY2C likely associated with heme metabolism based on gene clustering with hemC2C hemD in Proteobacteria (unrelated to HemY-type PPO in GramPositives)	-2.17	9.72E-08
1	1317162	1317950	-	Histidine ABC transporter2C ATP-binding protein HisP (TC 3.A.1.3.1)	-2.74	9.53E-06
1	1317975	1318688	-	Histidine ABC transporter2C permease protein HisM (TC 3.A.1.3.1)	-3.05	5.81E-05
1	1318698	1319387	-	Histidine ABC transporter2C permease protein HisQ (TC 3.A.1.3.1)	-3.12	8.65E-28
1	1319555	1320334	-	Lysine-arginine-ornithine-binding periplasmic protein precursor (TC 3.A.1.3.1)	-2.25	2.19E-31
1	1329210	1329698	-	Putative IpgF protein	-3.02	1.49E-08
1	1335458	1337131	+	FIG00453162: hypothetical protein	3.44	4.29E-14
1	1342348	1345065	-	Aconitate hydratase (EC 4.2.1.3) @ 2-methylisocitrate dehydratase (EC 4.2.1.99)	-4.80	6.09E-67
1	1345137	1346588	-	2-methylcitrate dehydratase (EC 4.2.1.79)	-2.69	1.23E-11
1	1346624	1347160	-	Probable signal peptide protein	-3.08	3.49E-06
1	1347270	1348484	-	2-methylcitrate synthase (EC 2.3.3.5)	2.17	9.84E-09
1	1348537	1349358	-	Methylisocitrate lyase (EC 4.1.3.30)	-2.76	3.09E-09
1	1356846	1358147	+	Citrate synthase (si) (EC 2.3.3.1)	-2.75	4.81E-119
1	1359076	1359312	+	Transcriptional regulator2C XRE family	3.65	1.87E-06
1	1377736	1378929	+	O-acetylhomoserine sulfhydrylase (EC 2.5.1.49) / O-succinylhomoserine sulfhydrylase (EC 2.5.1.48)	-2.33	4.35E-10
1	1385832	1388105	-	4-alpha-glucanotransferase (amylomaltase) (EC 2.4.1.25)	-2.36	6.34E-10
1	1390058	1392319	-	Glycogen debranching enzyme (EC 3.2.1.-)	-2.06	7.22E-06
1	1392420	1394636	-	12C4-alpha-glucan (glycogen) branching enzyme2C GH-13-type (EC 2.4.1.18)	-2.60	4.93E-09
1	1394629	1398120	-	Trehalose synthase (EC 5.4.99.16)	-3.37	1.17E-19
1	1398117	1401869	-	Alpha-amylase (EC 3.2.1.1)	-2.61	4.56E-07
1	1410699	1412369	+	Adenylate cyclase (EC 4.6.1.1)	6.09	0.00E+00
1	1413489	1414190	-	Membrane-associated phospholipid phosphatase	2.32	0.00E+00
1	1414502	1415680	+	Poly(3-hydroxybutyrate) depolymerase	2.17	0.00E+00
1	1415794	1415970	+	FIG00452784: hypothetical protein	7.87	9.44E-14
1	1416031	1416834	-	FIGfam005179	8.90	2.48E-15
1	1426035	1427393	+	NADH dehydrogenase (EC 1.6.99.3)	-2.08	1.91E-14
1	1434244	1435107	+	GTP cyclohydrolase I (EC 3.5.4.16) type 2	-2.01	1.35E-23
1	1438710	1440599	+	DNA primase (EC 2.7.7.-)	2.40	0.00E+00
1	1448293	1449189	-	3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.157)	2.92	3.95E-06
1	1449342	1450364	+	Ornithine utilization regulator	3.25	0.00E+00
1	1450785	1451750	+	ribose ABC transporter2C substrate-binding protein	2.41	5.34E-05
1	1453353	1455395	+	Ribose ABC transport system2C permease protein RbsC (TC 3.A.1.2.1)	4.12	0.00E+00

1	1455999	1456376	-	Homoserine kinase (EC 2.7.1.39)	2.32	6.31E-03
1	1465700	1466776	-	Putrescine transport ATP-binding protein PotA (TC 3.A.1.11.1)	2.59	2.04E-06
1	1466921	1467847	+	Glycine cleavage system transcriptional activator	3.36	1.37E-06
1	1467952	1469004	-	FOG: PAS/PAC domain	3.33	2.62E-08
1	1469137	1470219	-	monooxygenase2C putative	6.57	0.00E+00
1	1470607	1471791	+	3-ketoacyl-CoA thiolase (EC 2.3.1.16) @ Acetyl-CoA acetyltransferase (EC 2.3.1.9)	12.77	0.00E+00
1	1472152	1474344	+	Lead2C cadmium2C zinc and mercury transporting ATPase (EC 3.6.3.3) (EC 3.6.3.5)%3B Copper-translocating P-type ATPase (EC 3.6.3.4)	6.46	0.00E+00
1	1474703	1475866	+	ABC-transporter permease protein	3.81	6.11E-03
1	1477589	1478998	+	RND efflux system2C outer membrane lipoprotein CmeC	5.44	1.03E-03
1	1486223	1486639	+	Possible membrane protein	13.17	4.64E-09
1	1487903	1489183	-	Salicylate hydroxylase (EC 1.14.13.1)	-2.49	3.46E-03
1	1491920	1492849	+	22C3-dihydroxybiphenyl 12C2-dioxygenase	2.51	4.41E-03
1	1493794	1495251	+	Probable VANILLIN dehydrogenase oxidoreductase protein (EC 1.-.-.-)	3.31	9.40E-06
1	1495282	1496175	+	Acetoacetate decarboxylase (EC 4.1.1.4)	3.62	2.23E-05
1	1503633	1504391	+	Transcriptional regulatory protein ompR	2.79	1.53E-11
1	1554508	1555902	+	Selenium-binding protein 1	2.02	4.67E-03
1	1555927	1556559	+	hypothetical protein	3.25	1.36E-04
1	1556702	1557832	-	ABC transport system2C permease component YbhR	2.39	5.92E-04
1	1557847	1558776	-	ABC transport system2C permease component YbhR	2.98	2.84E-04
1	1558784	1559965	-	ABC transporter multidrug efflux pump2C fused ATP-binding domains	16.59	6.04E-06
1	1559962	1560900	-	ABC transporter multidrug efflux pump2C fused ATP-binding domains	13.18	2.13E-13
1	1560897	1561901	-	Predicted membrane fusion protein (MFP) component of efflux pump2C membrane anchor protein YbhG	10.89	0.00E+00
1	1562883	1563824	+	Permease of the drug/metabolite transporter (DMT) superfamily	2.83	4.88E-03
1	1563931	1564692	-	Propionate catabolism operon transcriptional regulator of GntR family [predicted]	3.15	1.81E-03
1	1572177	1572518	+	FIG00976088: hypothetical protein	8.14	3.54E-07
1	1572533	1573519	-	Ribose ABC transport system2C permease protein RbsC (TC 3.A.1.2.1)	3.45	2.36E-05
1	1574493	1576133	-	Ribose ABC transport system2C ATP-binding protein RbsA (TC 3.A.1.2.1)	3.25	3.57E-03
1	1576168	1577295	-	binding protein component precursor of ABC ribose transporter	6.52	7.70E-04
1	1577720	1578448	+	Transcriptional regulator PhnF	3.57	2.92E-07
1	1582847	1583617	-	Glycolate utilization operon transcriptional activator GlcC	4.88	0.00E+00
1	1585337	1586434	+	Glycolate dehydrogenase (EC 1.1.99.14)2C FAD-binding subunit GlcE	6.26	9.90E-07
1	1586451	1587695	+	Glycolate dehydrogenase (EC 1.1.99.14)2C iron-sulfur subunit GlcF	6.57	3.13E-10
1	1587697	1588101	+	Hypothetical protein GlcG in glycolate utilization operon	3.29	0.00E+00
1	1588148	1590322	+	Malate synthase G (EC 2.3.3.9)	2.69	2.43E-06
1	1590453	1592117	+	L-lactate permease	2.64	6.39E-08
1	1596028	1596786	-	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)	3.87	4.82E-05
1	1596825	1597610	-	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)	2.85	3.87E-03
1	1599024	1599905	-	Putative polysaccharide deacetylase	3.37	4.86E-15
1	1599928	1601271	-	Asp-tRNAAsn/Glu-tRNAGln amidotransferase A subunit and related amidases	4.66	2.43E-06
1	1601941	1604085	+	Acetone carboxylase2C beta subunit (EC 6.4.1.6) / N-methylhydantoinase A (EC 3.5.2.14)	22.72	5.22E-03

1	1604097	1606421	+	Acetone carboxylase2C alpha subunit (EC 6.4.1.6)	11.80	4.82E-08
1	1606463	1606969	+	Acetone carboxylase2C gamma subunit (EC 6.4.1.6)	10.66	3.03E-05
1	1607332	1609359	+	Sigma-54 dependent transcriptional activator	3.36	2.29E-11
1	1621504	1623237	+	Nitrogenase (molybdenum-iron)-specific transcriptional regulator NifA	2.93	7.19E-10
1	1639980	1640300	+	FIG00465754: hypothetical protein	-8.58	1.16E-04
1	1661090	1661857	+	Nitrate/nitrite sensor protein (EC 2.7.3.-)	14.59	3.32E-03
1	1670792	1671979	-	Sarcosine oxidase beta subunit (EC 1.5.3.1)	2.54	5.69E-03
1	1676418	1677326	+	Glutamine amidotransferase protein GlxB (EC 2.4.2.-)	3.75	5.77E-06
1	1677314	1678003	+	Glutamate synthase [NADPH] putative GlxC chain (EC 1.4.1.13)	3.34	3.69E-10
1	1678029	1679390	+	Glutamate synthase [NADPH] large chain (EC 1.4.1.13)	6.75	6.51E-03
1	1679525	1680910	+	Ammonium transporter	3.42	1.97E-12
1	1680957	1681643	-	Predicted regulator PutR for proline utilization2C GntR family	3.88	0.00E+00
1	1681913	1682509	+	FIG00953662: hypothetical protein	3.86	3.72E-09
1	1683449	1684405	+	Nitrilotriacetate monooxygenase component B (EC 1.14.13.-)	4.60	0.00E+00
1	1684476	1685519	+	Alkanal monooxygenase alpha chain (EC 1.14.14.3)	3.11	2.93E-03
1	1685571	1687043	+	Aldehyde dehydrogenase (EC 1.2.1.3)	2.19	4.07E-03
1	1687073	1687387	+	FIG00984243: hypothetical protein	2.77	1.28E-03
1	1699688	1700980	+	Asp-tRNAAsn/Glu-tRNAGln amidotransferase A subunit and related amidases	4.42	2.40E-11
1	1709851	1711146	-	metabolite/H symporter2C major facilitator superfamily (MFS)	-2.65	2.51E-21
1	1728265	1730187	+	VgrG protein	-2.47	1.61E-03
1	1745823	1746908	-	6-phosphogluconolactonase (EC 3.1.1.31)	3.26	3.44E-04
1	1750271	1751008	-	Transcriptional regulator2C GntR family	2.75	1.73E-06
1	1751151	1752890	-	Dihydroxy-acid dehydratase (EC 4.2.1.9)	9.09	4.72E-14
1	1753010	1754011	+	D-3-phosphoglycerate dehydrogenase (EC 1.1.1.95)	6.31	3.82E-05
1	1768464	1768862	+	Transcriptional regulator	3.85	2.47E-04
1	1771690	1772637	-	ABC transporter2C ATP-binding protein	10.05	8.52E-09
1	1772659	1773585	-	Thiamin biosynthesis lipoprotein ApbE	19.50	7.01E-05
1	1774181	1776151	+	Nitrous-oxide reductase (EC 1.7.99.6)	93.90	0.00E+00
1	1776260	1777516	+	Nitrous oxide reductase maturation protein NosD	5.57	7.28E-04
1	1777509	1778420	+	Nitrous oxide reductase maturation protein NosF (ATPase)	15.32	0.00E+00
1	1779247	1779774	+	Nitrous oxide reductase maturation protein2C outer-membrane lipoprotein NosL	11.71	0.00E+00
1	1779785	1780051	+	FIG00453002: hypothetical protein	7.13	0.00E+00
1	1780054	1780452	+	FIG00453671: hypothetical protein	8.63	9.61E-03
1	1784670	1786055	+	Ubiquinol--cytochrome c reductase2C cytochrome B subunit (EC 1.10.2.2)	3.74	2.11E-03
2	1796	2788	-	LysR family transcriptional regulator QseA	-2.26	9.69E-08
2	5288	5800	-	hypothetical protein	2.53	2.07E-12
2	11450	12730	-	Hexuronate transporter	2.57	1.59E-05
2	13237	14271	+	LysR family transcriptional regulator QseA	2.08	1.70E-04
2	25997	26248	+	hypothetical protein	12.61	9.72E-12
2	26270	26854	-	Predicted reductase RutE in novel pyrimidine catabolism pathway	4.43	0.00E+00
2	27044	27634	+	Alkylated DNA repair protein AlkB	2.39	6.86E-04
2	27677	28111	-	Universal stress protein UspA and related nucleotide-binding proteins	2.46	3.81E-04
2	28583	31810	+	miscellaneous%3B hypothetical/global homology	2.11	6.46E-03

2	36844	38532	-	Malonate decarboxylase alpha subunit	2.39	6.51E-04
2	38559	39251	-	Predicted regulator PutR for proline utilization2C GntR family	4.63	1.23E-10
2	39458	40765	+	Permeases of the major facilitator superfamily	2.96	9.23E-07
2	41017	41883	+	Arylamine N-acetyltransferase	2.19	7.81E-06
2	42338	43192	+	ABC-type amino acid transport/signal transduction systems2C periplasmic component/domain	7.27	0.00E+00
2	43337	44059	+	Histidine ABC transporter2C permease protein HisQ (TC 3.A.1.3.1)	11.29	2.39E-04
2	44074	44820	+	ABC-type arginine/histidine transport system2C permease component	12.40	0.00E+00
2	44891	45682	+	Histidine ABC transporter2C ATP-binding protein HisP (TC 3.A.1.3.1)	11.38	0.00E+00
2	45700	47376	+	Histidine ammonia-lyase (EC 4.3.1.3)	5.32	5.14E-07
2	47357	48241	+	Histidine utilization repressor	2.14	3.82E-03
2	57750	58754	-	putative Cytochrome bd22C subunit II	2.05	2.86E-05
2	63386	64972	-	Cyclohexanone monooxygenase (EC 1.14.13.22)	7.64	2.48E-15
2	74120	74623	+	hypothetical protein	-2.81	5.25E-07
2	75412	77004	-	FOG: TPR repeat	-2.22	1.66E-05
2	77036	77662	-	Uncharacterized glutathione S-transferase-like protein	-2.26	2.90E-68
2	86865	87203	+	hypothetical protein	2.97	4.80E-03
2	89477	90160	+	conserved hypothetical protein	6.54	7.06E-08
2	90553	90960	+	hypothetical protein	9.13	4.09E-08
2	122573	125890	+	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)	3.36	8.15E-05
2	128703	130661	-	Beta-galactosidase (EC 3.2.1.23)	6.63	2.06E-04
2	131780	132484	+	Ni2CFe-hydrogenase I cytochrome b subunit	4.14	1.06E-09
2	132493	132951	-	17 kDa surface antigen	67.16	8.42E-09
2	133082	133579	-	putative exported protein	467.66	1.72E-13
2	137610	138881	+	hypothetical protein	2.44	2.37E-10
2	138990	140447	-	Niacin transporter NiaP	3.05	1.05E-03
2	143995	144567	-	HTH-type transcriptional regulator BetI	2.33	6.81E-05
2	149016	150410	+	hypothetical protein	-2.25	3.63E-24
2	151321	153858	-	Ribonucleotide reductase of class II (coenzyme B12-dependent) (EC 1.17.4.1)	7.99	0.00E+00
2	154159	155253	+	FIG139552: Putative protease	45.57	0.00E+00
2	155259	156257	+	FIG139928: Putative protease	41.40	0.00E+00
2	156254	156724	+	hypothetical protein	28.24	0.00E+00
2	156771	157826	-	Transcriptional regulator containing an amidase domain and an AraC-type DNA-binding HTH domain	3.50	3.00E-10
3	3514	4776	-	Transcription termination factor Rho	-2.18	1.44E-31
3	31239	32423	+	3-ketoacyl-CoA thiolase (EC 2.3.1.16) @ Acetyl-CoA acetyltransferase (EC 2.3.1.9)	-2.67	7.52E-15
3	33524	34714	+	Cystathionine beta-lyase (EC 4.4.1.8)	-2.15	5.91E-05
3	46775	47227	-	hypothetical protein	2.43	7.84E-04
3	59129	59776	+	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (EC 2.7.7.60)	-2.48	1.22E-11
3	65650	65961	+	FIG00454707: hypothetical protein	2.03	1.47E-06
3	77918	78133	+	hypothetical protein	7.51	7.17E-04
3	79359	79988	+	hypothetical protein	2.11	5.33E-03
3	79985	80221	+	hypothetical protein	43.84	1.05E-05
3	81432	81737	+	Helicase subunit of the DNA excision repair complex	4.26	0.00E+00

3	82193	82378	+	hypothetical protein	3.77	6.14E-05
3	82390	82782	+	hypothetical protein	2.17	2.85E-03
3	85573	85854	+	hypothetical protein	3.41	2.48E-15
3	94641	97460	+	Phage protein	3.14	1.72E-09
3	106516	108909	+	Phage protein	2.10	4.03E-05
3	114626	115609	+	2-dehydropantoate 2-reductase (EC 1.1.1.169)	2.03	3.79E-03
3	118182	119528	+	Cell division trigger factor (EC 5.2.1.8)	-2.33	1.05E-28
3	129320	130945	-	Glucose-6-phosphate isomerase (EC 5.3.1.9)	-2.13	3.85E-07
3	138812	139591	-	Peptidyl-prolyl cis-trans isomerase (EC 5.2.1.8)	-3.05	2.83E-17
3	151673	152551	+	Short-chain dehydrogenase/reductase SDR	-3.22	2.46E-12
3	163327	164700	+	Alpha2Calpha-trehalose-phosphate synthase [UDP-forming] (EC 2.4.1.15)	-2.56	4.64E-14
3	164713	165948	+	putative RecF protein	-2.33	4.45E-07
3	166199	167266	+	Glycosyltransferase	2.07	9.99E-05
3	177683	178852	+	Polysaccharide export lipoprotein Wza	-9.97	3.51E-06
3	181180	182103	+	Glucosyl-3-phosphoglycerate synthase (EC 2.4.1.266)	4.05	5.16E-04
3	204025	206622	+	Type II/IV secretion system secretin RcpA/CpaC2C associated with Flp pilus assembly	2.39	2.24E-03
3	207052	208266	+	Type II/IV secretion system ATPase TadZ/CpaE2C associated with Flp pilus assembly	6.83	8.54E-03
3	215581	216111	-	hypothetical protein	4.97	1.40E-04
3	223567	224118	-	Response regulator	2.35	7.25E-04
3	224615	225043	-	Mobile element protein	6.49	1.95E-08
4	745	2061	-	Polysaccharide export lipoprotein Wza	2.56	1.87E-09
4	19075	19620	+	Cytochrome B561	7.01	5.81E-14
4	19639	21165	-	Acetyl-CoA hydrolase	5.95	0.00E+00
4	34330	35550	+	Outer membrane protein (porin)	-4.50	3.92E-14
4	37180	37437	-	hypothetical protein	-4.02	1.78E-11
4	37449	39188	-	Salicylate hydroxylase (EC 1.14.13.1)	-3.35	9.24E-20
4	44022	44198	+	hypothetical protein	4.14	5.42E-04
4	44220	45050	-	Hydroxyethylthiazole kinase (EC 2.7.1.50)	3.80	0.00E+00
4	45200	46078	-	Phosphogluconate repressor HexR2C RpiR family	3.17	6.35E-03
4	57647	58198	-	Iron-sulfur cluster regulator IscR	4.05	0.00E+00
4	58443	58697	-	hypothetical protein	2.09	1.50E-04
4	65079	66227	-	Outer membrane protein (porin)	2.78	7.27E-09
4	90502	91593	+	Immunogenic protein	3.14	2.93E-03
4	101438	102772	+	Gamma-glutamyl-putrescine synthetase (EC 6.3.1.11)	29.71	7.87E-03
4	102837	104243	+	Omega-amino acid--pyruvate aminotransferase (EC 2.6.1.18)	18.38	5.05E-03
4	104336	105457	+	Putrescine ABC transporter putrescine-binding protein PotF (TC 3.A.1.11.2)	13.96	4.20E-03
4	132228	133379	+	Alcohol dehydrogenase (EC 1.1.1.1)	37.66	7.16E-15
4	134713	136173	-	Predicted transcriptional regulator of pyridoxine metabolism	5.26	2.28E-05
4	136664	137131	+	Histone acetyltransferase HPA2 and related acetyltransferases	2.01	3.35E-07
4	143724	143954	+	hypothetical protein	-3.01	2.98E-06
4	145437	145985	+	FIG028593: membrane protein	3.28	6.11E-04
4	146142	147635	+	ATP synthase beta chain (EC 3.6.3.14)	21.76	0.00E+00
4	147644	148120	+	ATP synthase epsilon chain (EC 3.6.3.14)	51.63	0.00E+00

4	148752	149486	+	ATP synthase F0 sector subunit a	7.76	1.84E-04
4	149757	150509	+	ATP synthase F0 sector subunit b	6.11	6.24E-05
4	150514	152046	+	ATP synthase alpha chain (EC 3.6.3.14)	11.02	5.18E-03
4	159336	160487	+	Molybdenum cofactor biosynthesis protein MoaA	-2.10	8.39E-83
4	163189	163842	+	Response regulator containing a CheY-like receiver domain and an HTH DNA-binding domain	-2.17	4.20E-05
4	167266	168582	+	Nucleoside ABC transporter2C periplasmic nucleoside-binding protein	-2.17	3.19E-04
4	168546	169052	-	cytosolic long-chain acyl-CoA thioester hydrolase family protein	3.78	2.11E-06
4	169220	169717	-	hypothetical protein	-2.87	1.13E-24
4	169708	170220	-	Mannose-6-phosphate isomerase (EC 5.3.1.8)	-3.43	5.14E-09
4	181808	182887	+	Tenebrin	6.54	0.00E+00
4	182973	183527	-	FIG00553873: hypothetical protein	3.04	0.00E+00
4	184061	184174	+	hypothetical protein	5.77	2.83E-03
4	184242	184391	-	hypothetical protein	2.69	6.16E-03
4	187718	188380	+	Chromosome (plasmid) partitioning protein ParA	2.62	6.98E-06
4	189587	190939	-	Replication protein	-2.34	7.54E-11
4	196947	198146	+	2-amino-3-ketobutyrate coenzyme A ligase (EC 2.3.1.29)	5.19	0.00E+00
4	198164	199192	+	L-threonine 3-dehydrogenase (EC 1.1.1.103)	4.54	0.00E+00
4	199241	199978	-	Protein yceI precursor	4.15	0.00E+00
4	210342	210911	-	FIG00464676: hypothetical protein	-12.29	7.46E-03
4	214334	215569	+	Threonine dehydrogenase and related Zn-dependent dehydrogenases	4.20	4.16E-03
4	215863	217035	+	Cytochrome c oxidase polypeptide II (EC 1.9.3.1)	-5.55	8.67E-03
4	220538	222067	+	Putative diheme cytochrome c-553	-3.00	1.87E-03
4	251176	251298	-	hypothetical protein	-10.38	9.81E-04
4	262680	262964	+	FIG00456993: hypothetical protein	5.94	1.50E-09
4	263004	264215	-	Putative cytoplasmic protein clustered with trehalase	3.66	1.85E-08
4	268968	269513	+	Methylated-DNA--protein-cysteine methyltransferase (EC 2.1.1.63)	2.87	1.15E-03
4	269751	271412	-	Aerotaxis sensor receptor protein	3.83	0.00E+00
4	272911	274005	-	Lipase (EC 3.1.1.3)	2.25	1.84E-14
4	277603	278196	-	2-Keto-D-gluconate dehydrogenase (EC 1.1.99.4)2C membrane-bound2C gamma subunit	-2.73	8.50E-03
4	280346	281818	+	Protein containing domains DUF4042C DUF407	3.53	0.00E+00
4	281894	282904	+	Protein containing domains DUF403	2.64	2.24E-11
4	293308	294975	-	Manganese transport protein MntH	-2.39	3.78E-20
4	295034	295186	-	hypothetical protein	-2.95	1.52E-03
4	296207	297718	+	Carbohydrate-selective porin	-2.23	4.95E-07
4	299904	300179	+	FIG00455658: hypothetical protein	6.00	2.93E-05
4	300256	300588	+	hypothetical protein	7.23	1.05E-04
4	300666	300932	+	hypothetical protein	8.01	1.78E-12
4	300953	302356	-	D-beta-hydroxybutyrate permease	2.82	1.27E-13
4	302745	304235	+	Transcriptional regulator containing PAS2C AAA-type ATPase2C and DNA-binding domains	3.96	0.00E+00
4	304392	304988	+	FIG00452845: hypothetical protein	4.97	0.00E+00
4	305464	306306	-	Transcriptional regulatory protein	2.97	1.02E-04
4	306442	306555	+	hypothetical protein	10.89	1.90E-11
4	313825	316254	+	Protein acetyltransferase	2.42	6.39E-06

4	318187	318510	+	Altronate dehydratase (EC 4.2.1.7)	4.85	3.33E-07
4	320966	321859	+	L-fuconolactone hydrolase	2.18	2.21E-05
4	324426	326048	+	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	3.75	0.00E+00
4	327385	327558	+	hypothetical protein	7.38	1.76E-09
4	329742	330410	+	FIG00458480: hypothetical protein	2.23	6.23E-07
4	332696	332998	+	hypothetical protein	5.99	4.88E-04
4	334482	336512	-	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	4.82	0.00E+00
4	336787	339402	-	Lead2C cadmium2C zinc and mercury transporting ATPase (EC 3.6.3.3) (EC 3.6.3.5)%3B Copper-translocating P-type ATPase (EC 3.6.3.4)	9.67	1.93E-09
4	341372	341560	-	hypothetical protein	2.89	5.71E-05
4	345560	347350	-	Putative GntR-family regulatory protein and aminotransferase near polyamine transporter	3.38	0.00E+00
4	347491	348591	+	Putrescine transport ATP-binding protein PotA (TC 3.A.1.11.1)	4.62	1.06E-10
4	359001	359567	-	FIG00453502: hypothetical protein	2.04	3.55E-07
4	359824	361020	-	Response regulator containing CheY-like receiver2C AAA-type ATPase2C and DNA-binding domains	4.25	0.00E+00
4	361334	361675	+	Probable transmembrane protein	2.79	6.24E-05
4	361772	362035	+	FIG00457629: hypothetical protein	2.89	8.91E-04
4	362380	363783	+	Response regulator containing CheY-like receiver2C AAA-type ATPase2C and DNA-binding domains	2.72	0.00E+00
4	364313	364540	+	FIG00455317: hypothetical protein	3.54	7.42E-07
4	364725	366161	+	Alpha2Calpha-trehalose-phosphate synthase [UDP-forming] (EC 2.4.1.15)	-2.03	2.83E-10
4	368830	369312	-	Very-short-patch mismatch repair endonuclease (G-T specific)	3.41	8.12E-07
4	369367	369636	-	DNA-cytosine methyltransferase (EC 2.1.1.37)	4.44	2.23E-07
4	370475	372661	+	putative two-component system sensor histidine kinase2C putative heat shock protein	3.31	0.00E+00
4	373682	373852	+	hypothetical protein	7.23	0.00E+00
4	376757	377086	-	hypothetical protein	3.10	0.00E+00
4	377311	377553	-	hypothetical protein	2.50	2.46E-04
4	381878	383863	+	Topoisomerase IV subunit B (EC 5.99.1.-)	-2.67	1.26E-04
4	383932	386262	+	Topoisomerase IV subunit A (EC 5.99.1.-)	-3.03	1.33E-12
4	391372	392283	-	Phenazine biosynthesis protein PhzF like	2.03	7.20E-04
4	394135	395019	-	Permease of the drug/metabolite transporter (DMT) superfamily	2.80	2.18E-09
4	395097	395804	-	FIG00454405: hypothetical protein	4.37	4.90E-09
4	395925	396317	+	Transcriptional regulator2C GntR family domain / Aspartate aminotransferase (EC 2.6.1.1)	7.76	9.84E-06
4	396319	397428	+	Transcriptional regulator2C GntR family domain / Aspartate aminotransferase (EC 2.6.1.1)	5.16	2.75E-07
4	397510	399408	+	Chaperone protein HtpG	3.75	0.00E+00
4	400291	400791	+	G:T/U mismatch-specific uracil/thymine DNA-glycosylase	3.02	0.00E+00
4	401001	401285	-	hypothetical protein	3.93	1.99E-09
4	403206	404174	+	Spermidine synthase (EC 2.5.1.16)	-3.18	1.20E-03
4	411359	411724	+	Cytochrome c553	-2.90	1.41E-12
4	411771	412160	+	Cytochrome c553	-4.19	2.71E-04
4	414748	415380	+	Transcriptional regulator2C TetR family	2.53	7.06E-10
4	415473	416750	-	Intracellular PHB depolymerase (EC 3.1.1.-)	2.19	0.00E+00
4	419599	419751	-	hypothetical protein	2.65	1.93E-03
4	421438	422028	+	Lipid A acylation protein PagP2C palmitoyltransferase	-2.17	9.30E-04

4	422063	426058	-	Cell division protein FtsK	-2.24	1.25E-18
4	429060	429242	-	hypothetical protein	-9.62	8.51E-04
4	449223	449573	-	hypothetical protein	3.76	1.08E-08
4	449609	449767	-	hypothetical protein	4.09	3.81E-11
4	458888	459061	-	hypothetical protein	2.32	1.11E-03
4	479622	480206	-	Lysine decarboxylase family	2.61	0.00E+00
4	480274	480984	-	Transcriptional regulator2C TetR family	2.05	3.98E-05
4	483111	484079	-	Ser/Thr protein phosphatase family protein2C UDP-22C3-diacylglucosamine hydrolase (EC 3.6.1.-) homolog	3.94	6.57E-04
4	503725	504102	+	Preprotein translocase subunit SecG (TC 3.A.5.1.1)	2.63	7.03E-07
4	504457	504816	+	NADH ubiquinone oxidoreductase chain A (EC 1.6.5.3)	4.53	0.00E+00
4	505992	507245	+	NADH-ubiquinone oxidoreductase chain D (EC 1.6.5.3)	-2.58	3.48E-117
4	507389	507874	+	NADH-ubiquinone oxidoreductase chain E (EC 1.6.5.3)	-2.75	1.27E-204
4	507871	509199	+	NADH-ubiquinone oxidoreductase chain F (EC 1.6.5.3)	-3.56	7.80E-06
4	509390	511732	+	NADH-ubiquinone oxidoreductase chain G (EC 1.6.5.3)	-4.54	0.00E+00
4	511733	512797	+	NADH-ubiquinone oxidoreductase chain H (EC 1.6.5.3)	-6.49	2.53E-51
4	512813	513301	+	NADH-ubiquinone oxidoreductase chain I (EC 1.6.5.3)	-4.55	1.29E-05
4	514149	514454	+	NADH-ubiquinone oxidoreductase chain K (EC 1.6.5.3)	-7.60	2.69E-50
4	514472	516508	+	NADH-ubiquinone oxidoreductase chain L (EC 1.6.5.3)	-5.20	1.10E-29
4	516523	518013	+	NADH-ubiquinone oxidoreductase chain M (EC 1.6.5.3)	-8.09	1.26E-186
4	518039	519511	+	NADH-ubiquinone oxidoreductase chain N (EC 1.6.5.3)	-7.14	3.73E-12
4	519511	519807	+	FIG003089: Probable transmembrane protein	-5.01	1.51E-07
4	519860	520462	+	ADP-ribose pyrophosphatase (EC 3.6.1.13)	-3.86	2.36E-23
4	529519	531081	-	3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) / Enoyl CoA hydratase (EC 4.2.1.17)	2.61	0.00E+00
4	534630	535793	+	Predicted carboxypeptidase	-2.54	2.69E-07
4	536012	536551	-	Probable lipoprotein transmembrane	2.45	4.04E-05
4	536835	537518	-	INTEGRAL MEMBRANE PROTEIN (Rhomboid family)	2.25	1.82E-04
5	1243	1911	-	Hydrogenase-4 component E	2.37	1.91E-09
5	1912	2862	-	Formate hydrogenlyase subunit 4	3.67	2.86E-07
5	2859	4868	-	Hydrogenase-4 component B (EC 1.-.-.-) / Formate hydrogenlyase subunit 3	3.92	2.48E-15
5	5195	5692	+	FIG00455728: hypothetical protein	2.49	2.61E-07
5	6208	6405	+	Proline dehydrogenase	2.12	0.00E+00
5	6684	8591	+	Outer membrane protein A precursor	2.14	7.24E-03
5	9946	11331	-	Coproporphyrinogen III oxidase2C oxygen-independent (EC 1.3.99.22)	28.77	0.00E+00
5	17908	18132	-	hypothetical protein	2.23	0.00E+00
5	19273	20208	-	Ketopantoate reductase PanG (EC 1.1.1.169)	2.63	3.42E-11
5	20518	20679	+	FIG00452683: hypothetical protein	2.17	6.94E-08
5	21152	21445	+	Probable transmembrane protein	7.15	3.16E-03
5	21559	22242	-	Putative threonine efflux protein	3.48	0.00E+00
5	33260	33718	+	Methyltransferase type 11	2.63	1.34E-10
5	34707	36464	+	Sensor histidine kinase	2.63	0.00E+00
5	45350	46429	-	Urea ABC transporter2C urea binding protein	4.70	2.05E-04
5	52532	53530	+	Acetamidase (EC 3.5.1.4)	4.31	1.84E-13
5	57065	58156	-	Outer membrane protein (porin)	2.72	4.12E-05

5	58401	59522	-	Threonine dehydrogenase and related Zn-dependent dehydrogenases	12.96	1.46E-07
5	59524	60786	-	Major facilitator family transporter	8.58	7.16E-15
5	60853	61386	-	22C4'-dihydroxyacetophenone dioxygenase	16.31	2.23E-03
5	74880	75389	-	Mobile element protein	2.44	0.00E+00
5	92047	93042	-	Thioredoxin reductase (EC 1.8.1.9)	2.27	2.18E-08
5	105049	105582	+	Histone acetyltransferase HPA2 and related acetyltransferases	3.53	0.00E+00
5	124452	125750	-	Outer membrane protein (porin)	-3.44	1.72E-142
5	129369	129518	-	hypothetical protein	3.12	1.07E-04
5	139021	140037	-	XdhC protein (assists in molybdopterin insertion into xanthine dehydrogenase)	3.40	0.00E+00
5	140236	140412	-	hypothetical protein	2.87	0.00E+00
5	145018	146355	+	Para-aminobenzoate synthase2C amidotransferase component (EC 2.6.1.85)	2.22	5.31E-03
5	153020	156001	-	NAD-dependent formate dehydrogenase alpha subunit	2.81	4.39E-09
5	156016	157581	-	NAD-dependent formate dehydrogenase beta subunit	2.21	4.30E-03
5	157578	158117	-	NAD-dependent formate dehydrogenase gamma subunit	2.85	5.40E-06
5	161929	162579	-	Outer membrane protein A precursor	-2.02	5.67E-22
5	169156	170262	+	Biosynthetic Aromatic amino acid aminotransferase beta (EC 2.6.1.57)	-2.21	8.30E-09
5	170288	171331	+	Cyclohexadienyl dehydrogenase (EC 1.3.1.12)(EC 1.3.1.43)	-2.64	1.75E-07
5	171345	172649	+	5-Enolpyruvylshikimate-3-phosphate synthase (EC 2.5.1.19)	-3.14	1.99E-37
5	173542	175272	+	SSU ribosomal protein S1p	-2.34	2.72E-08
5	177484	178890	+	UDP-glucose dehydrogenase (EC 1.1.1.22)	-2.13	2.33E-07
5	179877	180869	+	ADP-L-glycero-D-manno-heptose-6-epimerase (EC 5.1.3.20)	-2.35	1.70E-30
5	188285	188938	+	Methionine ABC transporter permease protein	3.30	0.00E+00
5	189032	189829	+	Methionine ABC transporter substrate-binding protein	-2.15	1.71E-07
5	193828	195114	-	D-amino acid dehydrogenase small subunit (EC 1.4.99.1)	2.90	0.00E+00
5	195256	195744	+	Leucine-responsive regulatory protein2C regulator for leucine (or lrp) regulon and high-affinity branched-chain amino acid transport system	2.63	7.43E-09
5	200539	201222	+	Hypothetical nudix hydrolase YeaB	3.47	0.00E+00
5	201370	202305	+	Adenosylcobinamide-phosphate synthase (EC 6.3.1.10)	2.37	3.26E-09
5	205017	205649	+	3'-to-5' oligoribonuclease (orn)	3.09	5.81E-10
5	214092	215498	-	Fap amyloid fibril major component	3.73	4.91E-08
5	217630	217830	-	hypothetical protein	2.14	3.16E-05
5	217810	219201	+	Nitrogen regulation protein NR(I)	3.81	1.31E-04
5	220587	223295	-	FIG00457332: hypothetical protein	2.67	1.41E-04
5	224830	225333	+	Acyl-CoA hydrolase (EC 3.1.2.20)	-3.68	6.73E-15
5	230885	231016	-	hypothetical protein	2.81	1.56E-03
5	242803	244287	+	DNA recombination protein RmuC	-3.01	1.88E-08
5	247225	248343	-	Molybdenum cofactor biosynthesis protein MoaA	2.05	0.00E+00
5	248629	251904	-	Ribonuclease E (EC 3.1.26.12)	-2.11	4.41E-17
5	254422	254814	+	Ferredoxin2C 2Fe-2S	2.53	1.28E-07
5	254832	255845	+	Periplasmic serine proteases (ClpP class)	2.26	2.02E-12
5	261984	262733	+	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)	-2.87	4.03E-04
5	262899	263138	+	Acyl carrier protein	-2.29	2.61E-23
5	289149	289721	-	Cytochrome oxidase biogenesis protein Sco1/SenC/PrrC2C putative copper metallochaperone	-3.22	2.55E-13

5	300648	301424	+	Predicted D-glucarate or D-galactorate regulator2C GntR family	3.93	0.00E+00
5	301495	302442	-	5-dehydro-4-deoxyglucarate dehydratase (EC 4.2.1.41)	5.16	2.69E-07
5	302567	303559	-	Ribose operon repressor	5.01	0.00E+00
5	303844	304677	+	UDP-glucose 4-epimerase (EC 5.1.3.2)	8.62	9.34E-07
5	319984	321192	-	FIG00461923: hypothetical protein	3.45	6.75E-03
5	321274	321459	-	hypothetical protein	15.73	0.00E+00
5	321478	322728	+	Glycosyltransferase	2.80	5.29E-03
5	332577	334517	+	Cell division protein FtsH (EC 3.4.24.-)	93.39	2.85E-08
5	334775	335968	+	Ferredoxin	29.33	0.00E+00
5	335955	336413	+	cAMP-binding proteins - catabolite gene activator and regulatory subunit of cAMP-dependent protein kinases	8.83	3.89E-09
5	336400	337281	+	2-polyprenylphenol hydroxylase and related flavodoxin oxidoreductases	7.87	5.59E-07
5	337283	338086	+	hydrogenase/sulfur reductase2C delta subunit	23.85	6.59E-06
5	338076	339365	+	Coenzyme F420-reducing hydrogenase2C alpha subunit	6.76	8.08E-07
5	339879	340166	+	hypothetical protein	22.94	3.44E-12
5	349964	351070	+	surface antigen	2.08	1.10E-04
5	362266	364026	-	Formylmethanofuran dehydrogenase subunit A (EC 1.2.99.5)	4.87	5.23E-03
5	364023	365405	-	Formylmethanofuran dehydrogenase subunit B (EC 1.2.99.5)	8.44	4.21E-04
5	366660	367766	+	FIG00858196: hypothetical protein	3.96	9.88E-03
5	369005	369595	+	delta 1-pyrroline-5-carboxylate synthetase	12.37	0.00E+00
5	369558	370301	-	duf556 family protein	3.83	9.79E-03
5	370298	370933	-	DUF447 family protein	7.72	0.00E+00
5	371334	371537	-	hypothetical protein	8.35	0.00E+00
5	373085	373201	+	hypothetical protein	11.49	5.58E-03
5	380923	382305	-	Related to Dihydropteroate synthase	2.06	5.32E-04
5	382284	382883	-	Archaeal flavoprotein COG1036	4.04	0.00E+00
5	382880	384967	-	Transcriptional activator of acetoin/glycerol metabolism	3.07	0.00E+00
5	385257	385859	+	Dihydroneopterin aldolase (EC 4.1.2.25)	4.77	0.00E+00
5	386087	386530	-	FIG00453204: hypothetical protein	4.31	5.28E-12
5	386712	387221	-	Formaldehyde activating enzyme	2.88	6.42E-03
5	392724	393749	-	beta-ribofuranosylaminobenzene 5'-phosphate synthase	6.02	4.45E-04
5	394980	396806	+	Methanol dehydrogenase large subunit protein (EC 1.1.99.8)	4.64	5.88E-09
5	400815	403031	-	Beta-glucosidase (EC 3.2.1.21)	3.14	0.00E+00
5	414130	414687	-	Transcriptional regulator2C TetR family	-2.41	3.21E-03
5	415368	416933	+	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	2.63	8.02E-06
5	417126	417860	-	Transcriptional regulator2C GntR family	4.57	0.00E+00
5	418099	419124	+	ABC transporter2C substrate binding protein	6.12	0.00E+00
5	419155	420756	+	Ribose ABC transport system2C ATP-binding protein RbsA (TC 3.A.1.2.1)	8.47	0.00E+00
5	422804	423727	+	Gluconolactonase (EC 3.1.1.17)	3.12	2.10E-03
5	440647	442200	+	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	2.97	1.42E-04
5	442251	442454	+	hypothetical protein	9.30	5.29E-04
5	470907	471692	-	UPF0028 protein YchK	2.31	8.42E-05
5	479551	481008	+	D-aminoacylase (EC 3.5.1.81)	2.74	8.72E-03
5	505226	505699	-	FIG00985911: hypothetical protein	25.49	0.00E+00

5	512354	513997	+	two-component system sensor kinase	2.71	9.65E-14
5	529422	529889	+	Outer membrane protein (porin)	2.40	5.84E-11
5	541389	543014	+	Permeases of the major facilitator superfamily	2.20	4.90E-09
5	543078	543668	-	Autoinducer synthesis protein LuxI	2.20	2.42E-06
5	544132	544860	-	Transcriptional activator protein LuxR	2.82	1.14E-07
5	553970	555712	+	Dihydroxy-acid dehydratase (EC 4.2.1.9)	2.07	1.96E-04
5	556351	557280	+	Serine acetyltransferase (EC 2.3.1.30)	3.00	5.73E-11
5	557300	558121	+	Transcriptional regulator2C AraC family	2.17	1.33E-07
5	558248	559237	+	Permease of the drug/metabolite transporter (DMT) superfamily	2.21	1.32E-03
5	560070	560993	-	LysR-family transcriptional regulator	5.69	0.00E+00
5	561131	562321	+	P-hydroxybenzoate hydroxylase (EC 1.14.13.2)	7.39	0.00E+00
5	569437	570846	+	Transcriptional regulator2C GntR family domain / Aspartate aminotransferase (EC 2.6.1.1)	2.78	4.56E-03
5	572759	573373	-	Transporter2C LysE family	7.65	3.34E-10
5	573497	575260	-	Arsenical pump-driving ATPase (EC 3.6.3.16)	8.07	0.00E+00
5	575270	576115	-	Arsenical resistance operon trans-acting repressor ArsD	5.57	1.13E-13
5	576134	576664	-	Cytochrome c family protein	4.89	2.39E-06
5	576684	577754	-	Arsenical-resistance protein ACR3	5.08	1.17E-06
5	577777	578280	-	Arsenate reductase (EC 1.20.4.1)	8.58	0.00E+00
5	578285	578758	-	Lactoylglutathione lyase (EC 4.4.1.5) @ Cadmium-induced protein CadI	14.46	1.92E-11
5	578771	579094	-	Transcriptional regulator2C ArsR family	18.56	2.75E-04
5	590585	590821	+	hypothetical protein	2.19	7.14E-04
5	591854	592474	+	Outer membrane lipoprotein	2.15	4.62E-08
5	592566	593051	+	FIG00456008: hypothetical protein	6.78	1.97E-06
5	597029	597469	+	histone-like protein	-10.63	2.38E-05
5	598683	599951	-	Outer membrane protein (porin)	-7.00	2.18E-17
5	600230	601657	-	Ribonuclease BN (EC 3.1.-.-)	8.78	0.00E+00
5	601728	602075	-	hypothetical protein	-2.18	1.26E-06
5	615459	622610	+	Fibronectin type III domain protein	-2.51	3.45E-06
5	622727	624226	+	Outer membrane efflux protein precursor	-4.40	4.98E-27
5	624238	625068	+	Membrane-fusion protein	4.61	3.46E-11
5	625083	626546	+	Membrane-fusion protein	-2.66	5.37E-04
5	633934	634872	+	Ethanolamine operon regulatory protein	6.92	5.11E-04
5	644420	644812	-	hypothetical protein	4.15	1.53E-03
5	645072	646259	+	Outer membrane protein (porin)	11.77	0.00E+00
5	646630	647781	-	Leucine-2C isoleucine-2C valine-2C threonine-2C and alanine-binding protein	8.55	6.40E-13
5	647892	648731	-	putative dioxygenase	13.36	1.52E-13
5	648741	650060	-	FAD dependent oxidoreductase	28.26	0.00E+00
5	650176	651252	-	Cystathionine beta-lyase (EC 4.4.1.8)	34.69	0.00E+00
5	651849	652547	-	Chemotaxis response - phosphatase CheZ	4.38	5.82E-13
5	653949	655622	+	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	5.76	1.22E-06
5	655639	656181	+	Positive regulator of CheA protein activity (CheW)	3.79	6.96E-05
5	657355	658164	-	Chemotaxis protein methyltransferase CheR (EC 2.1.1.80)	11.80	4.00E-13
5	658223	660244	-	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	3.02	5.69E-04

5	660297	660830	-	Positive regulator of CheA protein activity (CheW)	3.39	1.66E-07
5	660849	662921	-	Signal transduction histidine kinase CheA (EC 2.7.3.-)	5.99	8.75E-09
5	662918	663241	-	hypothetical protein	2.61	2.71E-03
5	663645	664808	-	Methyl-accepting chemotaxis protein	5.34	3.88E-11
5	665477	666523	+	Serine-pyruvate aminotransferase/archaeal aspartate aminotransferase	2.30	3.37E-08
5	666615	667502	-	Nucleoside-diphosphate-sugar epimerases	2.67	1.79E-06
5	667601	668476	+	Putative DNA-binding protein in cluster with Type I restriction-modification system	2.76	2.56E-06
5	668551	669138	+	Integral membrane protein	2.39	2.74E-09
5	670565	671725	+	FIG00453175: hypothetical protein	2.69	1.40E-04
5	687208	688845	+	Salicylate hydroxylase (EC 1.14.13.1)	-2.37	7.23E-07
5	694328	695047	-	Two-component response regulator	3.00	2.31E-05
5	731769	732605	+	Transcriptional regulator2C IclR family	2.98	1.87E-03
5	759862	760374	-	General secretion pathway protein C	3.63	4.81E-03
5	772042	773190	+	FOG: TPR repeat	9.30	3.83E-03
5	773320	773961	+	Adenylylsulfate kinase (EC 2.7.1.25)	3.59	0.00E+00
5	791718	792599	+	FIG146518: Zn-dependent hydrolases2C including glyoxylases	2.76	0.00E+00
5	792647	794302	+	FIG003847: Oxidoreductase (flavoprotein)	8.13	0.00E+00
5	795255	796019	+	FIG135464: Cytochrome c4	4.32	3.04E-07
5	797742	798998	-	3-hydroxyphenylpropionic acid transporter	4.50	1.96E-04
5	800757	801350	-	Thiosulfate reductase cytochrome B subunit (membrane anchoring protein)	2.97	8.02E-05
5	856003	856830	+	4-hydroxycinnamoyl CoA hydratase/lyase (Enoyl-CoA hydratase/lyase) (EC 4.2.1.17)	2.99	8.80E-07
5	856894	858345	+	Probable VANILLIN dehydrogenase oxidoreductase protein (EC 1.-.-.-)	2.47	2.70E-06
5	860431	862293	+	Acyl-CoA dehydrogenase (EC 1.3.8.7)	7.70	5.75E-03
5	862404	863363	-	Flavodoxin reductases (ferredoxin-NADPH reductases) family 1%3B Vanillate O-demethylase oxidoreductase (EC 1.14.13.-)	24.09	0.00E+00
5	863501	864547	-	Probable vanillate O-demethylase oxygenase subunit oxidoreductase protein (EC 1.14.13.-)	23.82	0.00E+00
5	864675	865442	-	Transcriptional regulator for ferulate or vanillate catabolism	25.64	0.00E+00
5	879345	882131	-	ABC-type multidrug transport system2C permease component	9.27	5.76E-04
5	882141	883214	-	Putative membrane protein	37.70	6.19E-09
5	883211	884704	-	RND efflux system2C outer membrane lipoprotein CmeC	33.39	0.00E+00
5	885025	885912	+	ABC-type molybdate transport system2C periplasmic component	3.07	0.00E+00
5	971978	972892	+	LysR family transcriptional regulator Bucepa02002399	11.60	0.00E+00
5	991828	992121	+	hypothetical protein	2.10	9.40E-04
5	994570	995217	-	Transcriptional regulator2C GntR family	4.59	0.00E+00
5	995234	996601	-	Aminotransferase class-III	5.28	1.75E-11
5	996587	996712	+	hypothetical protein	15.03	0.00E+00
5	1017381	1017602	+	hypothetical protein	12.42	3.18E-11
5	1017619	1018311	+	Transcriptional regulator2C GntR family	12.37	1.12E-09
5	1018374	1019252	-	Succinyl-CoA ligase [ADP-forming] alpha chain (EC 6.2.1.5)	32.74	4.29E-05
5	1019254	1020450	-	Succinyl-CoA ligase [ADP-forming] beta chain (EC 6.2.1.5)	25.31	2.81E-07
5	1020808	1022568	+	Acetolactate synthase large subunit (EC 2.2.1.6)	109.82	4.71E-10
5	1022606	1023853	+	Formyl-coenzyme A transferase (EC 2.8.3.16)	58.03	4.07E-09
5	1023954	1026233	+	Acetyl-CoA synthetase (ADP-forming) alpha chain (EC 6.2.1.13)	30.01	2.21E-06

5	1026374	1027189	+	Fumarylacetoacetate hydrolase family protein	8.41	1.36E-04
5	1027318	1027629	-	FIG00454455: hypothetical protein	37.56	0.00E+00
5	1027726	1028673	-	Cys regulon transcriptional activator CysB	2.34	2.62E-03
5	1029002	1030393	+	Permeases of the major facilitator superfamily	7.17	6.07E-03
5	1033271	1034173	-	2-dehydropantoate 2-reductase (EC 1.1.1.169)	2.09	3.40E-03
5	1034147	1034368	+	hypothetical protein	4.14	6.28E-03
5	1039436	1040038	+	Phosphohydrolase (MutT/nudix family protein)	-3.23	3.87E-03
5	1040221	1041630	+	6-phosphogluconate dehydrogenase2C decarboxylating (EC 1.1.1.44)	-3.10	3.73E-35
5	515981	516388	+	Probable lipoprotein	4.93	2.86E-04
5	1061898	1062356	-	PhnG protein	2.66	2.05E-06
5	1064816	1065631	+	Non-heme chloroperoxidase (EC 1.11.1.10)	-2.35	9.62E-03
5	1089003	1089986	+	Transcriptional regulator2C DeoR family	10.67	1.25E-11
5	1090069	1091517	-	Aldehyde dehydrogenase B (EC 1.2.1.22)	6.58	7.47E-05
5	1091574	1092869	-	Gamma-aminobutyrate:alpha-ketoglutarate aminotransferase (EC 2.6.1.19)	6.52	3.97E-07
5	1092891	1093010	+	hypothetical protein	4.98	5.37E-03
5	1093028	1094659	+	Transcriptional regulator GabR of GABA utilization (GntR family with aminotransferase-like domain)	4.92	4.18E-07
5	1094833	1095678	-	Permeases of the drug/metabolite transporter (DMT) superfamily	5.26	4.17E-09
5	1101770	1102408	+	PE_PGRS family protein	97.56	1.62E-04
5	1106914	1111788	+	NAD-specific glutamate dehydrogenase (EC 1.4.1.2)2C large form	3.60	3.19E-12
5	1124949	1126508	+	RND efflux system2C outer membrane lipoprotein CmeC	3.56	4.80E-05
5	1126626	1127321	-	Two-component response regulator	5.20	4.50E-04
5	1128310	1128630	-	Flagellar transcriptional activator FlhD	3.60	0.00E+00
5	1129170	1129988	-	hypothetical protein	6.80	7.16E-15
5	1130061	1130306	-	hypothetical protein	2.69	1.31E-09
5	1132757	1133821	+	Deoxyribose-phosphate aldolase (EC 4.1.2.4)	2.48	2.49E-05
5	1139067	1140068	+	ABC transporter2C periplasmic sugar-binding protein precursor	-3.87	1.06E-03
5	1144256	1144525	+	hypothetical protein	4.65	2.75E-11
5	1144556	1145980	-	Permeases of the major facilitator superfamily	2.35	3.05E-03
5	1148876	1149922	+	4-hydroxyproline epimerase (EC 5.1.1.8)	2.78	1.84E-05
5	1149919	1151058	+	D-amino-acid oxidase (EC 1.4.3.3)	3.55	7.59E-03
5	1151356	1152651	+	Putative oxidoreductase in 4-hydroxyproline catabolic gene cluster	5.48	1.00E-02
5	1154984	1156180	-	Outer membrane porin protein 32 precursor%3B putative 3-hydroxyphenylpropionic acid porine	7.08	7.03E-11
5	1156377	1158311	-	4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27)	46.09	2.53E-07
5	1158621	1159076	+	3-dehydroquinate dehydratase II (EC 4.2.1.10)	8.19	0.00E+00
5	1159073	1159927	+	Quinate/shikimate 5-dehydrogenase I delta (EC 1.1.1.25)	15.72	0.00E+00
5	1160056	1161396	+	D-glucarate permease	2.61	1.48E-08
5	1162355	1163623	-	Lysine-specific permease	8.84	4.83E-09
5	1163995	1167144	+	Membrane carboxypeptidase (penicillin-binding protein)	14.71	0.00E+00
5	1167246	1168511	-	Transcriptional regulator FrcR for fructose utilization2C ROK family	37.70	0.00E+00
5	1168574	1169371	-	Fructose ABC transporter2C ATP-binding component FrcA	2.29	1.48E-04
5	1169439	1170431	-	Fructose ABC transporter2C permease component FrcC	3.01	2.51E-03
5	1173123	1174595	+	Hydantoin permease	2.31	9.98E-07

5	1178644	1179825	+	Isovaleryl-CoA dehydrogenase (EC 1.3.8.4)%3B Butyryl-CoA dehydrogenase (EC 1.3.99.2)	-2.17	6.34E-56
5	1179894	1181501	+	Methylcrotonyl-CoA carboxylase carboxyl transferase subunit (EC 6.4.1.4)	-2.49	3.28E-25
5	1181575	1182363	+	Methylglutaconyl-CoA hydratase (EC 4.2.1.18)	-2.90	8.82E-19
5	1182490	1184514	+	Methylcrotonyl-CoA carboxylase biotin-containing subunit (EC 6.4.1.4)	-3.55	5.53E-23
5	1184618	1185409	-	ABC-type phosphate/phosphonate transport system2C periplasmic component	-2.49	4.19E-03
5	1185428	1186507	-	Fatty acid desaturase	-3.40	4.66E-03
5	1186838	1187962	-	Sensory box histidine kinase	2.68	0.00E+00
5	1196589	1197368	-	Predicted L-lactate dehydrogenase2C Fe-S oxidoreductase subunit YkgE	2.40	6.19E-03
5	1197510	1198313	+	Glycolate utilization operon transcriptional activator GlcC	2.71	1.45E-04
5	1204598	1205323	+	Putative alkanesulfonate metabolism utilization regulator	3.31	0.00E+00
5	1209069	1211399	+	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)	2.19	6.43E-03
5	1211966	1212928	-	PTS IIA-like nitrogen-regulatory protein PtsN	2.99	8.97E-04
5	1214507	1215415	-	Glycine cleavage system transcriptional activator	3.46	0.00E+00
5	1215514	1216449	+	Permease of the drug/metabolite transporter (DMT) superfamily	2.31	6.48E-04
5	1216617	1217711	+	NmrA-like	2.43	2.18E-12
5	1220437	1220886	-	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)	4.70	0.00E+00
5	1222036	1224219	-	Phospholipase C 4 precursor (EC 3.1.4.3)	23.69	0.00E+00
5	1224294	1224494	-	hypothetical protein	6.25	8.92E-06
5	1226515	1227222	+	Succinate dehydrogenase iron-sulfur protein (EC 1.3.99.1)	7.51	7.44E-08
5	1245492	1246946	-	Indoleacetamide hydrolase (EC 3.5.1.-)	3.29	1.90E-10
5	1249538	1249891	-	hypothetical protein	3.76	1.55E-12
5	1249933	1250319	-	hypothetical protein	2.14	1.24E-09
5	1250454	1251050	+	hypothetical protein	8.19	1.49E-03
5	1251135	1251536	+	hypothetical protein	4.52	1.73E-06
5	1251623	1252747	-	Ribose ABC transport system2C permease protein RbsC (TC 3.A.1.2.1)	4.17	4.14E-06
5	1252744	1253781	-	Putative sugar ABC transport system2C permease protein YtfT	3.63	9.87E-04
5	1255309	1256274	-	Putative sugar ABC transport system2C periplasmic binding protein YtfQ precursor	3.98	1.51E-06
5	1256763	1258505	+	L-arabonate dehydratase (EC 4.2.1.25)	3.88	3.42E-05
5	1259493	1259618	+	hypothetical protein	3.52	1.78E-06
5	1259671	1260954	+	FIG00459198: hypothetical protein	3.09	8.69E-06
5	1278569	1279273	+	hypothetical protein	2.31	7.36E-04
5	1280361	1281131	+	Short-chain dehydrogenase/reductase SDR	3.79	3.06E-03
5	1284976	1285470	-	hypothetical protein	-2.31	5.71E-09
5	1304544	1305119	-	Response regulator NasT	2.24	3.39E-03
5	1305133	1307940	-	Assimilatory nitrate reductase large subunit (EC:1.7.99.4)	11.01	1.63E-10
5	1312690	1312908	-	FIG00461189: hypothetical protein	17.03	5.03E-03
5	1313972	1314388	-	Ferredoxin	3.48	9.07E-03
5	1318070	1318363	+	hypothetical protein	3.64	4.22E-09
5	1320057	1320833	-	Putative stomatin/prohibitin-family membrane protease subunit aq_911	8.32	1.14E-05
5	1320830	1322467	-	Putative membrane-bound ClpP-class protease associated with aq_911	7.62	2.54E-04
5	1323854	1324987	+	Butyryl-CoA dehydrogenase (EC 1.3.99.2)	-3.17	3.56E-09
5	1326934	1328481	+	Methylmalonate-semialdehyde dehydrogenase (EC 1.2.1.27)	-5.09	1.43E-06

5	1342126	1343091	+	Regulatory protein of benzoate catabolism	4.44	2.44E-11
5	1348815	1349858	-	Flagellar motor rotation protein MotA	4.57	2.78E-10
5	1352096	1352812	-	Transcriptional regulator2C PadR family	3.01	5.33E-08
5	1356090	1357004	-	Glutaminase (EC 3.5.1.2)	-4.27	6.96E-03
5	1357269	1358057	+	Transcriptional regulator2C AraC family	3.71	8.54E-10
5	1359878	1360063	-	hypothetical protein	2.52	3.50E-05
5	1362370	1363176	+	Alpha/beta hydrolase fold (EC 3.8.1.5)	3.26	6.12E-07
5	1368344	1369693	+	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase (EC 2.6.1.62)	2.17	8.07E-09
5	1369837	1370391	+	Cation/multidrug efflux pump	2.82	4.39E-06
5	1370617	1371042	+	Osmotically inducible protein C	2.96	6.55E-03
5	1371405	1372187	+	Probable carboxyvinyl-carboxyphosphonate phosphorylmutase (EC 2.7.8.23)	4.31	1.25E-03
5	1372348	1372461	-	hypothetical protein	5.41	4.36E-05
5	1372708	1372989	+	hypothetical protein	4.58	1.17E-14
5	1373051	1373554	-	Histone acetyltransferase HPA2 and related acetyltransferases	3.23	7.00E-10
5	1379129	1380499	+	PE_PGRS family protein	7.50	5.83E-05
5	1380602	1382605	-	FOG: PAS/PAC domain	2.62	1.48E-05
5	1393099	1395243	-	Signal transduction response regulator / Tetratricopeptide repeat-containing protein	-2.85	1.66E-06
5	1423694	1428517	-	Lhr-like helicases	7.38	0.00E+00
5	1440847	1441971	+	Universal stress protein UspA and related nucleotide-binding proteins	59.46	0.00E+00
5	1442027	1442500	+	Universal stress protein UspA and related nucleotide-binding proteins	170.76	1.61E-07
5	1442497	1442760	+	hypothetical protein	75.64	9.24E-10
5	1442803	1443939	+	ABC transporter2C permease protein	61.43	0.00E+00
5	1443950	1444666	+	Methionine ABC transporter ATP-binding protein	49.12	3.33E-08
5	1444663	1445760	+	membrane protein2C putative	12.64	3.25E-04
5	1445816	1447201	+	Outer membrane protein	34.17	2.25E-10
5	1447504	1447788	+	hypothetical protein	93.18	8.58E-11
5	1447884	1449548	-	Predicted kinase	70.83	2.55E-09
5	1449642	1452182	-	alternate gene name: yzbB	25.27	0.00E+00
5	1452248	1453417	-	COG1306 predicted glycoside hydrolase	12.30	1.13E-06
5	1453723	1454601	-	Polysaccharide deacetylase	24.66	0.00E+00
5	1454927	1455808	+	Osmotically inducible protein Y precursor	107.15	2.41E-08
5	1455983	1456495	+	Asp-tRNAAsn/Glu-tRNAGln amidotransferase B subunit (PET112 homolog)	67.29	0.00E+00
5	1456543	1458594	+	Cell division protein FtsH (EC 3.4.24.-)	78.02	1.39E-06
5	1458581	1460416	+	DNA polymerase X family	469.15	1.90E-06
5	1460545	1461663	+	FIG00460500: hypothetical protein	56.07	0.00E+00
5	1461624	1462763	-	Ribose-phosphate pyrophosphokinase (EC 2.7.6.1)	69.40	3.87E-05
5	1462763	1462888	-	hypothetical protein	16.31	1.36E-05
5	1462910	1463803	+	hypothetical protein	41.58	8.92E-13
5	1464032	1464691	-	Oxygen-insensitive NADPH nitroreductase (EC 1.-.-.-)	81.78	0.00E+00
5	1465147	1465797	+	Osmotically inducible protein Y precursor	41.83	0.00E+00
5	1465947	1466240	+	hypothetical protein	110.19	4.96E-04
5	1466332	1466622	-	hypothetical protein	186.35	0.00E+00
5	1466854	1467303	+	CBS domain protein	23.55	3.95E-06

5	1467423	1468331	-	Carbamate kinase (EC 2.7.2.2)	76.33	9.79E-04
5	1468398	1469396	-	Ornithine carbamoyltransferase (EC 2.1.3.3)	149.45	1.12E-05
5	1469528	1470745	-	Arginine deiminase (EC 3.5.3.6)	135.42	1.35E-05
5	1470761	1472251	-	Arginine/ornithine antiporter ArcD	30.11	1.44E-13
5	1473003	1476038	-	Long-chain-fatty-acid--CoA ligase (EC 6.2.1.3)	11.09	0.00E+00
5	1476182	1477099	-	Decarboxylase family protein	14.68	2.08E-11
5	1477421	1477732	+	Cation transport ATPase	139.30	3.39E-11
5	1477849	1478217	-	FIG00460628: hypothetical protein	160.59	0.00E+00
5	1478252	1478626	-	Cytochrome c551/c552	112.56	0.00E+00
5	1478876	1479907	-	Oxygen-insensitive NADPH nitroreductase (EC 1.-.-.-)	241.61	8.38E-08
5	1480082	1481068	+	Alcohol dehydrogenase (EC 1.1.1.1)	4.14	6.39E-13
5	1484521	1485039	-	Transcriptional regulator2C GntR family domain / Aspartate aminotransferase (EC 2.6.1.1)	2.75	9.71E-03
5	1485422	1486144	+	Predicted Fe-S-cluster oxidoreductase	6.27	1.67E-06
5	1486379	1487185	-	transcriptional regulator2C Crp/Fnr family	8.61	0.00E+00
5	1487195	1487503	-	FOG: CheY-like receiver	9.82	0.00E+00
5	1491258	1491722	+	Universal stress protein UspA and related nucleotide-binding proteins	85.26	2.26E-04
5	1492293	1493060	+	probable vgr related protein	4.03	5.87E-08
5	1493057	1495003	+	FIG027190: Putative transmembrane protein	2.99	1.19E-13
5	1498456	1499655	-	FIG005478: Porin2C Gram-negative type	3.68	2.73E-06
5	1504383	1505375	+	Transcriptional regulator2C LysR family	2.70	1.09E-05
5	1506287	1506460	+	hypothetical protein	19.75	0.00E+00
5	1509212	1510075	-	Universal stress protein UspA and related nucleotide-binding proteins	53.05	4.18E-11
5	1510072	1510614	-	Universal stress protein UspA and related nucleotide-binding proteins	65.99	5.41E-04
5	1510685	1511977	-	Acetate kinase (EC 2.7.2.1)	14.89	1.35E-03
5	1511993	1512832	-	Universal stress protein UspA and related nucleotide-binding proteins	58.34	2.80E-10
5	1513266	1513916	+	hypothetical protein	115.63	3.83E-10
5	1513906	1514652	+	Acetoacetyl-CoA reductase (EC 1.1.1.36)	92.19	0.00E+00
5	1514649	1516577	+	Polyhydroxyalkanoic acid synthase	10.70	1.23E-05
5	1516574	1517617	+	Phosphate acetyltransferase (EC 2.3.1.8)	20.33	1.54E-08
5	1517856	1518290	+	Molecular chaperone (small heat shock protein)	43.54	1.92E-06
5	1518579	1519976	-	Metallo-beta-lactamase family protein2C RNA-specific	58.79	0.00E+00
5	1520223	1520765	+	Flavodoxin	62.81	5.21E-07
5	1520785	1521246	+	CBS domain protein	57.83	3.10E-04
5	1521427	1523478	+	FIG00460868: hypothetical protein	9.66	3.33E-04
5	1525496	1525747	+	hypothetical protein	12.45	2.03E-04
5	1526511	1527902	-	Intracellular PHB depolymerase (EC 3.1.1.-)	40.63	3.03E-11
5	1527921	1528268	-	FIG00454208: hypothetical protein	44.82	0.00E+00
5	1528352	1528660	-	hypothetical protein	4.79	5.16E-07
5	1529417	1531840	+	Phosphoenolpyruvate synthase (EC 2.7.9.2)	15.61	6.17E-08
5	1531861	1532868	+	Tagatose-6-phosphate kinase (EC 2.7.1.144) / 1-phosphofructokinase (EC 2.7.1.56)	19.72	6.67E-06
5	1532869	1533564	-	Nitrate/nitrite response regulator protein	6.20	2.01E-06
5	1533561	1535438	-	Nitrate/nitrite sensor protein (EC 2.7.3.-)	7.40	0.00E+00
5	1535618	1536139	-	hypothetical protein	9.60	0.00E+00

5	1536763	1537581	-	Peptidyl-prolyl cis-trans isomerase PpiD (EC 5.2.1.8)	52.73	0.00E+00
5	1537604	1538320	-	Respiratory nitrate reductase gamma chain (EC 1.7.99.4)	28.68	0.00E+00
5	1538317	1539072	-	Respiratory nitrate reductase delta chain (EC 1.7.99.4)	36.32	1.22E-06
5	1539075	1540598	-	Respiratory nitrate reductase beta chain (EC 1.7.99.4)	26.48	7.33E-10
5	1540626	1544432	-	Respiratory nitrate reductase alpha chain (EC 1.7.99.4)	25.04	2.74E-06
5	1545878	1547155	-	Nitrate/nitrite transporter	2.95	6.90E-11
5	1547566	1548021	+	hypothetical protein	2.33	1.51E-04
5	1548236	1548643	+	putative two-component system response regulator	4.58	8.94E-05
5	1548830	1549279	+	CBS domain protein	222.94	0.00E+00
5	1549563	1550879	+	Acyl-CoA dehydrogenase%3B probable dibenzothiophene desulfurization enzyme	7.75	1.63E-06
5	1552168	1553622	+	Coenzyme F420-dependent N52CN10-methylene tetrahydromethanopterin reductase and related flavin-dependent oxidoreductases	3.87	1.83E-05
5	1553788	1555233	+	Major facilitator superfamily (MFS) transporter	2.66	1.65E-03
5	1555797	1558667	+	ClpB protein	59.97	0.00E+00
5	1558742	1559455	-	Predicted D-glucarate or D-galactorate regulator2C GntR family	9.71	0.00E+00
5	1559783	1561333	+	Possible fucose ABC transporter2C ATP-binding component	2.43	7.42E-03
5	1561326	1562357	+	Ribose ABC transport system2C permease protein RbsC (TC 3.A.1.2.1)	2.17	8.39E-03
5	1571474	1573231	-	Sulfate transporter	13.82	4.72E-14
5	1581837	1582841	+	L-arabinose-binding periplasmic protein precursor AraF (TC 3.A.1.2.2)	2.10	2.46E-04
5	1586983	1588362	+	Sensory box histidine kinase	15.93	8.48E-04
5	1595846	1597483	-	L-aspartate oxidase (EC 1.4.3.16)	4.71	1.73E-03
5	1597491	1598159	-	Ferredoxin:4Fe-4S ferredoxin2C iron-sulfur binding	5.26	7.35E-04
5	1598559	1600571	+	Signal transduction histidine kinase regulating C4-dicarboxylate transport system (EC 2.7.13.3)	13.79	5.71E-07
5	1600565	1601890	+	C4-dicarboxylate transport transcriptional regulatory protein	10.62	9.70E-03
5	1602329	1603990	+	NAD-dependent malic enzyme (EC 1.1.1.38)	4.28	5.51E-04
5	1606509	1607312	+	D-beta-hydroxybutyrate dehydrogenase (EC 1.1.1.30)	3.31	5.56E-03
5	1607354	1608151	+	Transcriptional regulator2C AraC family	3.07	2.45E-06
5	1608444	1609784	+	cytochrome C	20.00	0.00E+00
5	1609800	1610447	+	Cytochrome c4	32.86	8.42E-04
5	1610458	1611996	-	FOG: GGDEF domain	5.46	5.07E-10
5	1637422	1638270	+	2-hydroxymuconic semialdehyde hydrolase (EC 3.7.1.9)	15.57	1.17E-14
5	1647335	1648030	+	Predicted transcriptional regulator of N-Acetylglucosamine utilization2C GntR family	3.38	3.41E-14
5	1648059	1649333	+	5-methylthioribose kinase (EC 2.7.1.100)	4.21	2.91E-09
5	1649380	1650450	+	Methylthioribose-1-phosphate isomerase (EC 5.3.1.23)	2.53	2.93E-03
5	1656826	1658286	-	Mobile element protein	2.89	0.00E+00
5	1658379	1658591	-	N-acetyl-L2CL-diaminopimelate deacetylase (EC 3.5.1.47)	9.35	2.62E-04
5	1658645	1659286	-	Transporter2C LysE family	3.31	0.00E+00
5	1659402	1659869	+	Leucine-responsive regulatory protein2C regulator for leucine (or lrp) regulon and high-affinity branched-chain amino acid transport system	4.13	0.00E+00
5	1661055	1662365	+	Permeases of the major facilitator superfamily	3.68	3.62E-08
5	1662415	1663716	+	Choline-sulfatase (EC 3.1.6.6)	4.53	3.66E-13
5	1664219	1666903	-	Cation-transporting ATPase2C E1-E2 family	6.00	1.79E-08
5	1667019	1667180	-	hypothetical protein	2.41	4.23E-03

5	1668847	1668966	-	hypothetical protein	2.46	7.25E-13
5	1669181	1669882	+	hypothetical protein	8.84	7.81E-06
5	1678470	1680173	-	FIG004684: SpoVR-like protein	15.06	0.00E+00
5	1680170	1681441	-	FIG002076: hypothetical protein	16.53	0.00E+00
5	1681602	1683524	-	Serine protein kinase (prkA protein)2C P-loop containing	15.71	0.00E+00
5	1683637	1683768	-	hypothetical protein	4.24	3.87E-03
5	1685201	1685422	+	hypothetical protein	2.61	8.15E-07
5	1694351	1694908	-	FIG00460418: hypothetical protein	2.77	6.17E-05
5	1695249	1695830	+	Phosphate starvation-inducible protein PhoH2C predicted ATPase	3.39	1.52E-05
5	1695937	1697034	-	Carboxynorspermidine dehydrogenase	3.99	5.05E-08
5	1698016	1698501	+	2-polyprenylphenol hydroxylase and related flavodoxin oxidoreductases	3.31	0.00E+00
5	1701599	1702567	-	2-ketogluconate 6-phosphate reductase (EC 1.1.1.43)	2.84	3.46E-04
5	1703997	1705115	-	2-ketogluconate kinase (EC 2.7.1.13)	3.42	1.37E-04
5	1705123	1705947	-	Epimerase KguE	5.05	1.36E-04
5	1709598	1711331	+	Chloride channel protein	-2.33	1.02E-03
5	1713343	1713618	+	hypothetical protein	2.63	2.20E-07
5	1713726	1713956	+	Superfamily II DNA and RNA helicases	3.50	5.42E-04
5	1719455	1720954	+	RND efflux system2C outer membrane lipoprotein2C NodT family	-3.71	1.34E-04
5	1732775	1732930	-	hypothetical protein	2.98	2.51E-14
5	1735576	1736394	-	Putrescine transport system permease protein PotI (TC 3.A.1.11.2)	-4.14	1.17E-28
5	1736391	1737323	-	Putrescine transport system permease protein PotH (TC 3.A.1.11.2)	-4.41	7.11E-08
5	1737320	1738483	-	Putrescine transport ATP-binding protein PotG (TC 3.A.1.11.2)	-3.45	2.08E-57
5	1738610	1739710	-	Putrescine ABC transporter putrescine-binding protein PotF (TC 3.A.1.11.2)	-3.62	8.36E-04
5	1740672	1741346	-	Outer membrane protein W precursor	41.26	0.00E+00
5	1741414	1742673	-	Dioxygenases related to 2-nitropropane dioxygenase	8.83	0.00E+00
5	1742915	1743430	-	Probable lipoprotein	12.06	0.00E+00
5	1743430	1745301	-	Protein ImpG/VasA	8.06	0.00E+00
5	1745298	1748897	-	probable membrane protein YPO1482	11.17	0.00E+00
5	1748929	1750230	-	hypothetical protein	9.96	0.00E+00
5	1750230	1750490	-	FIG00461523: hypothetical protein	39.77	1.40E-14
5	1751247	1753736	-	FIG00977738: hypothetical protein	4.91	0.00E+00
5	1759898	1760107	+	FIG00453532: hypothetical protein	9.95	1.79E-05
5	1765674	1766606	+	Transcriptional regulator2C LysR family	2.01	0.00E+00
5	1766735	1768258	-	Guanine deaminase (EC 3.5.4.3)%3B Hydroxydechloroatrazine ethylaminohydrolase (EC 3.5.99.3)	4.30	6.46E-04
5	1770944	1771456	-	Ureidoglycolate hydrolase (EC 3.5.3.19)	3.11	3.44E-04
5	1788408	1789406	-	Transcriptional regulator containing an amidase domain and an AraC-type DNA-binding HTH domain	3.72	3.43E-04
5	1789861	1790787	+	L-proline glycine betaine binding ABC transporter protein ProX (TC 3.A.1.12.1)	4.05	1.27E-03
5	1794689	1794853	+	hypothetical protein	5.30	0.00E+00
5	1796212	1796505	-	FIG00459104: hypothetical protein	6.23	7.25E-04
5	1802457	1803278	+	Membrane proteins related to metalloendopeptidases	3.78	0.00E+00
5	1803328	1804518	-	2-methylaconitate cis-trans isomerase	2.59	4.24E-06
5	1804551	1807148	-	2-methylcitrate dehydratase FeS dependent (EC 4.2.1.79)	2.72	4.70E-13

5	1807535	1807930	-	2-methylcitrate synthase (EC 2.3.3.5)	7.01	9.49E-05
5	1807959	1808849	-	Methylisocitrate lyase (EC 4.1.3.30)	10.27	0.00E+00
5	1809169	1811226	+	Propionate catabolism operon regulatory protein PrpR	4.52	3.76E-06
5	1826873	1828225	-	Uncharacterized protein ImpJ/VasE	3.64	2.61E-05
5	1831019	1833355	+	VgrG protein	9.87	0.00E+00
5	1851978	1852772	+	DNA-3-methyladenine glycosylase II (EC 3.2.2.21)	2.96	6.55E-03
5	1853020	1853358	+	hypothetical protein	7.34	1.28E-09
5	1853612	1854052	-	Inosine-5'-monophosphate dehydrogenase (EC 1.1.1.205)	5.02	2.75E-08
5	1859597	1860340	-	Transcriptional regulator2C IclR family	6.35	2.15E-11
5	1865576	1865836	+	hypothetical protein	7.73	0.00E+00
5	1865882	1865998	+	hypothetical protein	2.17	0.00E+00
5	1872765	1873457	+	Multiple antibiotic resistance protein marC	-2.31	5.83E-05
5	1873485	1875368	-	Two-component hybrid sensor and regulator	-2.98	3.03E-21
5	1884026	1885897	-	Trehalase (EC 3.2.1.28)	-2.06	7.49E-03
5	1888926	1889681	+	FIG00456996: hypothetical protein	-2.68	1.17E-04
5	1889936	1892341	-	Multimodular transpeptidase-transglycosylase (EC 2.4.1.129) (EC 3.4.-.-)	-2.23	1.99E-07
5	1892356	1898481	-	Large extracellular alpha-helical protein	-2.15	1.58E-16
5	1898818	1899879	+	HoxN/HupN/NixA family nickel/cobalt transporter	2.49	6.89E-14
5	1900051	1900368	-	Sigma 54 modulation protein YhbH	3.82	2.48E-15
5	1900680	1901075	+	DnaK suppressor protein	3.80	5.92E-04
5	1901359	1901778	-	Glycine-rich cell wall structural protein precursor	2.62	7.74E-05
5	1902047	1903588	-	Deoxyribodipyrimidine photolyase (EC 4.1.99.3)	3.07	0.00E+00
5	1913334	1913663	+	L-rhamnose mutarotase	-2.24	3.35E-04
5	1913827	1914063	-	hypothetical protein	53.97	0.00E+00
5	1914073	1914447	+	hypothetical protein	51.52	0.00E+00
5	1914466	1916937	+	ATP-dependent protease La (EC 3.4.21.53) Type II	16.41	7.16E-15
5	1917137	1917514	+	miscellaneous%3B unknown	94.31	7.80E-03
5	1921708	1922352	+	3'-5' exonuclease domain similar to epsilon subunit of DNA polymerase III2C PA3232-type	4.10	2.53E-03
6	12052	13545	+	Anthranilate synthase2C aminase component (EC 4.1.3.27)	-2.69	4.22E-10
6	13559	14152	+	Anthranilate synthase2C amidotransferase component (EC 4.1.3.27) @ Para-aminobenzoate synthase2C amidotransferase component (EC 2.6.1.85)	-3.78	1.62E-07
6	14152	15201	+	Anthranilate phosphoribosyltransferase (EC 2.4.2.18)	-3.33	9.54E-33
6	15250	16035	+	Indole-3-glycerol phosphate synthase (EC 4.1.1.48)	-3.58	1.28E-37
6	16063	16716	+	Adenylate cyclase (EC 4.6.1.1)	-3.23	5.18E-09
6	16750	17595	+	Uracil-DNA glycosylase2C family 1	-2.85	2.07E-05
6	21376	22548	-	2-octaprenyl-3-methyl-6-methoxy-12C4-benzoquinol hydroxylase (EC 1.14.13.-)	2.08	1.40E-05
6	34128	34397	-	FIG00452780: hypothetical protein	2.89	2.33E-09
6	40455	42047	-	Amino acid transporter	-2.11	1.63E-03
6	42926	44095	-	Putative heat shock protein YegD	-2.11	1.14E-09
6	54844	56550	-	Two-component hybrid sensor and regulator	-3.48	4.22E-04
7	1087900	1088835	+	Leader peptidase (Prepilin peptidase) (EC 3.4.23.43) / N-methyltransferase (EC 2.1.1.-)	2.04	3.11E-06
7	1101828	1102580	-	Cell division protein FtsQ	-3.23	9.06E-51
7	1102633	1103571	-	D-alanine--D-alanine ligase (EC 6.3.2.4)	-2.37	3.58E-03
7	1103568	1104965	-	UDP-N-acetylmuramate--alanine ligase (EC 6.3.2.8)	-3.12	5.88E-09

7	1104962	1106086	-	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase (EC 2.4.1.227)	-3.42	3.80E-22
7	1107351	1108874	-	UDP-N-acetylmuramoylalanine--D-glutamate ligase (EC 6.3.2.9)	-2.15	3.25E-19
7	1118055	1119266	+	Outer membrane protein (porin)	-2.84	8.57E-06
7	132225	133730	+	Aldehyde dehydrogenase (EC 1.2.1.3)	3.45	1.13E-04
7	141775	142716	-	Gentisate 12C2-dioxygenase (EC 1.13.11.4)	-2.21	2.15E-03
7	156240	157913	+	Enoyl-CoA hydratase (EC 4.2.1.17) / Delta(3)-cis-delta(2)-trans-enoyl-CoA isomerase (EC 5.3.3.8) / 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) / 3-hydroxybutyryl-CoA epimerase (EC 5.1.2.3)	3.04	8.93E-04
7	159856	160824	+	Flavodoxin reductases (ferredoxin-NADPH reductases) family 1%3B Vanillate O-demethylase oxidoreductase (EC 1.14.13.-)	2.63	0.00E+00
7	167163	168257	-	Outer membrane protein (porin)	2.38	2.30E-03
7	168967	169944	-	Putative esterase	2.08	5.00E-03
7	14480	17734	+	Carbamoyl-phosphate synthase large chain (EC 6.3.5.5)	-2.13	1.15E-35
7	170597	170902	-	Ferredoxin	6.32	0.00E+00
7	171232	171963	+	Paralog of coenzyme PQQ synthesis protein C	3.75	2.82E-03
7	174109	175134	+	CDP-6-deoxy-delta-32C4-glucoseen reductase-like	3.68	1.79E-09
7	191445	192338	-	Hca operon (3-phenylpropionic acid catabolism) transcriptional activator HcaR	2.21	5.44E-11
7	197438	198982	-	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	3.26	8.27E-06
7	204942	205853	-	LysR-family transcriptional regulator	3.05	0.00E+00
7	215937	217454	+	Indolepyruvate oxidoreductase subunit IorB II (EC 1.2.7.8)	12.90	4.15E-11
7	20571	22466	+	Cell division protein FtsH (EC 3.4.24.-)	2.15	1.44E-05
7	217887	220217	+	probable bifunctional hydroxylase/oxidoreductase	4.09	2.83E-03
7	232333	234126	+	Two-component hybrid sensor and regulator	3.20	0.00E+00
7	234131	235843	+	Nitrogen regulation protein NR(I)	3.22	3.38E-10
7	239874	241037	+	hypothetical protein	3.55	0.00E+00
7	242852	244078	+	INTEGRAL MEMBRANE PROTEIN (Rhomboid family)	2.28	1.94E-07
7	25175	26206	+	Phosphate ABC transporter2C periplasmic phosphate-binding protein PstS (TC 3.A.1.7.1)	4.64	0.00E+00
7	26379	27383	+	Phosphate transport system permease protein PstC (TC 3.A.1.7.1)	4.85	2.48E-15
7	264780	266195	-	Transcriptional regulator2C GntR family domain / Aspartate aminotransferase (EC 2.6.1.1)	31.99	0.00E+00
7	27380	28276	+	Phosphate transport system permease protein PstA (TC 3.A.1.7.1)	2.40	0.00E+00
7	272544	273887	+	monooxygenase2C putative	12.77	9.08E-04
7	274498	275688	+	Outer membrane protein (porin)	5.99	0.00E+00
7	285769	287019	+	cAMP-binding proteins - catabolite gene activator and regulatory subunit of cAMP-dependent protein kinases	10.26	4.69E-04
7	291834	292982	-	Probable Co/Zn/Cd efflux system membrane fusion protein	2.14	6.41E-06
7	297615	297755	-	hypothetical protein	19.25	0.00E+00
7	297802	298458	+	Predicted phosphoribosyltransferases	302.09	0.00E+00
7	298677	298862	-	hypothetical protein	5.39	7.14E-03
7	298997	299308	+	FIG00461068: hypothetical protein	4.79	2.13E-03
7	327390	328940	+	Histidine ammonia-lyase (EC 4.3.1.3)	13.62	0.00E+00
7	328945	329640	+	Histidine utilization repressor	18.54	0.00E+00
7	329767	331455	+	Urocanate hydratase (EC 4.2.1.49)	7.65	0.00E+00
7	331490	332089	+	Conserved hypothetical protein (perhaps related to histidine degradation)	6.56	0.00E+00

7	332165	333418	+	Imidazolonepropionase (EC 3.5.2.7)	5.07	2.18E-11
7	333381	333497	+	hypothetical protein	2.56	6.63E-04
7	333494	334879	+	Formiminoglutamic iminohydrolase (EC 3.5.3.13)	2.98	3.56E-05
7	342995	343780	-	FIG00459494: hypothetical protein	-4.90	6.78E-05
7	2150	2629	-	hypothetical protein	9.48	4.55E-04
7	355570	356991	+	Fe-S oxidoreductase	-2.61	4.79E-65
7	36115	36933	-	Putative hemolysin	4.21	1.63E-10
7	367192	368439	+	Succinylornithine transaminase (EC 2.6.1.81)	2.73	1.50E-11
7	376157	377482	-	diguanylate cyclase (GGDEF domain) with PAS/PAC sensor	-2.20	4.57E-04
7	377479	378852	-	putative ATPase	-2.33	1.96E-35
7	37871	39142	-	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	4.44	1.04E-05
7	390807	394130	-	Acriflavin resistance plasma membrane protein	-4.29	6.90E-17
7	394134	397250	-	Cobalt-zinc-cadmium resistance protein CzcA%3B Cation efflux system protein CusA	-3.93	3.37E-26
7	397308	398501	-	Probable Co/Zn/Cd efflux system membrane fusion protein	-3.04	5.95E-05
7	399130	399321	+	hypothetical protein	-2.17	2.93E-08
7	401726	403156	+	Signal transduction histidine kinase	-2.07	8.03E-05
7	403178	403858	+	Response regulators consisting of a CheY-like receiver domain and a winged-helix DNA-binding domain	-2.66	5.50E-08
7	39250	40632	-	Type I secretion outer membrane protein2C TolC precursor	18.05	0.00E+00
7	40675	40884	-	Rhodanese-related sulfurtransferase	49.22	4.45E-05
7	419953	420981	+	FIG00455389: hypothetical protein	8.64	5.13E-06
7	420992	423970	+	Glycolate dehydrogenase (EC 1.1.99.14)2C subunit GlcD	3.17	0.00E+00
7	424305	425456	+	Leucine-2C isoleucine-2C valine-2C threonine-2C and alanine-binding protein	4.46	4.39E-04
7	40895	44143	-	Acriflavin resistance protein	18.94	2.48E-15
7	433385	433723	+	FIG00453397: hypothetical protein	2.80	2.15E-03
7	44160	45182	-	Efflux transporter2C RND family2C MFP subunit2C AcrA/E family	96.97	3.44E-06
7	437959	438486	+	Acetyltransferase (EC 2.3.1.-)	4.17	1.12E-05
7	45301	45414	+	hypothetical protein	158.30	2.67E-07
7	451111	454869	+	Exodeoxyribonuclease V beta chain (EC 3.1.11.5)	-2.36	6.09E-06
7	454911	457019	+	Exodeoxyribonuclease V alpha chain (EC 3.1.11.5)	-3.34	4.11E-07
7	459255	459512	-	DNA-binding protein H-NS	4.32	3.70E-05
7	459835	460086	-	FIG00453023: hypothetical protein	2.38	5.56E-03
7	461438	461752	+	FIG00454649: hypothetical protein	2.01	4.21E-03
7	461949	462221	-	FIG00453881: hypothetical protein	3.00	1.16E-04
7	45671	46051	+	Transcriptional regulator2C ArsR family	3.49	4.94E-05
7	462565	462966	-	Transposase and inactivated derivatives	3.09	3.16E-06
7	474600	474968	+	Ribosome-binding factor A	-2.69	5.14E-04
7	46182	46610	-	FIG00456299: hypothetical protein	4.78	2.45E-06
7	474980	475933	+	tRNA pseudouridine synthase B (EC 4.2.1.70)	-2.42	4.62E-10
7	476038	477597	-	Inner membrane component of tripartite multidrug resistance system	-2.08	1.13E-18
7	477673	478908	-	Membrane fusion component of tripartite multidrug resistance system	-2.13	1.33E-14
7	483831	486686	+	2-oxoglutarate dehydrogenase E1 component (EC 1.2.4.2)	-2.63	8.94E-25
7	486806	488089	+	Dihydrolipoamide succinyltransferase component (E2) of 2-oxoglutarate dehydrogenase complex (EC 2.3.1.61)	-3.66	1.94E-08
7	488217	489647	+	Dihydrolipoamide dehydrogenase of 2-oxoglutarate	-4.58	1.99E-25

				dehydrogenase (EC 1.8.1.4)		
7	489793	490890	+	ATPase component BioM of energizing module of biotin ECF transporter	-6.51	1.29E-18
7	497545	497727	+	Flp pilus assembly protein2C pilin Flp	2.97	7.51E-14
7	498366	498941	+	Flp pilus assembly protein TadG	-2.13	2.50E-04
7	47106	49754	+	two-component hybrid sensor and regulator	3.57	4.73E-03
7	499931	501361	+	Type II/IV secretion system secretin RcpA/CpaC2C associated with Flp pilus assembly	-2.59	6.75E-05
7	502736	504163	+	Type II/IV secretion system ATP hydrolase TadA/VirB11/CpaF2C TadA subfamily	-3.78	3.50E-06
7	508010	509698	+	Probable transmembrane protein	-4.41	1.05E-05
7	49858	51075	+	mRNA 3-end processing factor	2.66	3.41E-03
7	509708	511099	+	Sigma-54 dependent transcriptional regulator	-3.82	1.05E-06
7	512051	512227	-	hypothetical protein	3.73	4.25E-07
7	512658	512861	+	hypothetical protein	41.21	3.20E-12
7	51072	52802	+	ATP-dependent DNA ligase (EC 6.5.1.1) LigC	2.59	6.35E-09
7	53067	53891	+	RecA/RadA recombinase	3.04	6.98E-10
7	539184	541625	-	4-hydroxythreonine-4-phosphate dehydrogenase (EC 1.1.1.262)	4.53	5.03E-04
7	541622	542791	-	Alcohol dehydrogenase (EC 1.1.1.1)	5.89	4.50E-07
7	542884	543771	-	4-hydroxy-tetrahydrodipicolinate synthase (EC 4.3.3.7)	8.68	8.49E-13
7	543814	544962	-	Outer membrane protein (porin)	9.41	6.06E-10
7	561200	563986	-	Beta-mannosidase (EC 3.2.1.25)	4.10	3.02E-03
7	3369	4703	-	Probable oxidoreductase	3.14	3.22E-04
7	585582	586301	+	Transcriptional regulators	14.04	1.17E-13
7	60315	60845	+	Transcriptional regulator2C MerR family	3.16	0.00E+00
7	602796	603023	+	hypothetical protein	3.82	3.35E-07
7	603596	603874	-	FIG00455658: hypothetical protein	7.22	5.02E-11
7	604366	605319	+	tRNA dihydrouridine synthase A	3.40	1.77E-06
7	608529	608822	+	hypothetical protein	3.62	1.92E-04
7	608859	609413	-	hypothetical protein	2.79	6.51E-10
7	610246	610443	+	hypothetical protein	2.38	7.57E-06
7	612387	612647	+	hypothetical protein	3.84	2.56E-11
7	617393	617575	+	hypothetical protein	2.19	0.00E+00
7	617811	617993	+	hypothetical protein	2.86	5.01E-03
7	618812	620815	+	Phage protein	2.41	1.36E-03
7	620818	620979	+	hypothetical protein	8.82	2.63E-04
7	628776	629201	+	hypothetical protein	-2.02	3.49E-03
7	633164	633589	+	T4-like phage baseplate hub + tail lysozyme	6.78	1.82E-03
7	636164	637639	+	hypothetical protein	11.93	0.00E+00
7	644530	645420	+	Glutamate Aspartate periplasmic binding protein precursor GltI (TC 3.A.1.3.4)	5.44	8.14E-10
7	66371	67570	-	Aromatic-amino-acid aminotransferase (EC 2.6.1.57)	-2.75	4.34E-24
7	653462	653932	+	Universal stress protein UspA and related nucleotide-binding proteins	7.61	0.00E+00
7	653978	654667	-	FIG00454323: hypothetical protein	2.66	0.00E+00
7	654664	655014	-	FIG00453151: hypothetical protein	2.54	1.64E-09
7	655042	655692	-	SOS-response repressor and protease LexA (EC 3.4.21.88)	2.06	3.47E-04
7	659356	660414	+	Sulfate and thiosulfate import ATP-binding protein CysA (EC 3.6.3.25)	2.90	4.86E-03

7	663920	664714	-	Hydroxypyruvate isomerase (EC 5.3.1.22)	2.85	8.52E-07
7	664760	666526	-	Glyoxylate carboligase (EC 4.1.1.47)	4.77	1.11E-06
7	4964	6115	+	hypothetical protein	2.34	2.15E-05
7	670518	670817	+	Primosomal replication protein N	-2.06	5.92E-04
7	670820	671095	+	SSU ribosomal protein S18p @ SSU ribosomal protein S18p2C zinc-independent	-2.06	2.07E-11
7	70457	71014	+	Periplasmic protein p19 involved in high-affinity Fe2+ transport	-5.25	8.79E-05
7	677257	677424	+	hypothetical protein	2.04	8.94E-03
7	677403	679259	-	Predicted ATPase related to phosphate starvation-inducible protein PhoH	3.39	0.00E+00
7	679225	679365	+	hypothetical protein	2.84	0.00E+00
7	683180	684247	-	Polymyxin resistance protein ArnC2C glycosyl transferase (EC 2.4.-.-)	-2.20	3.12E-15
7	684486	685646	-	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase (EC 2.6.1.-)	-2.10	4.38E-05
7	688253	688627	-	membrane protein2C putative	2.03	0.00E+00
7	690378	691715	+	Homoserine dehydrogenase (EC 1.1.1.3)	-2.04	1.44E-16
7	695004	695477	+	Molybdenum cofactor biosynthesis protein MoaE	-2.15	4.98E-05
7	71478	72320	+	High-affinity iron permease	-3.20	7.58E-07
7	695525	696091	-	Truncated hemoglobins	2.11	4.69E-08
7	697068	699665	+	ClpB protein	13.16	0.00E+00
7	699806	700234	-	FIG00454267: hypothetical protein	8.45	1.04E-09
7	700441	701214	-	Flagellar hook-length control protein FliK	3.58	8.74E-12
7	72317	73738	+	Ferredoxin	-2.91	1.77E-10
7	705103	705882	+	Formate hydrogenlyase subunit 3/Multisubunit Na+/H+ antiporter2C MnhD subunit	4.57	1.07E-08
7	713079	713267	+	FOG: TPR repeat	9.21	0.00E+00
7	717708	718412	-	Branched-chain amino acid transport ATP-binding protein LivF (TC 3.A.1.4.1)	-2.73	1.06E-03
7	718424	719200	-	Branched-chain amino acid transport ATP-binding protein LivG (TC 3.A.1.4.1)	-2.35	2.20E-04
7	721623	721910	-	hypothetical protein	2.46	6.51E-04
7	722850	723809	-	4-hydroxy-3-methylbut-2-enyl diphosphate reductase (EC 1.17.1.2)	2.51	1.24E-04
7	724582	725226	+	DNA repair protein RadC	2.31	3.47E-03
7	75126	75524	-	Predicted redox protein2C regulator of disulfide bond formation	2.10	6.28E-03
7	736253	739090	+	Isoleucyl-tRNA synthetase (EC 6.1.1.5)	-2.24	9.18E-54
7	739691	740905	+	Phosphopantothenoylcysteine decarboxylase (EC 4.1.1.36) / Phosphopantothenoylcysteine synthetase (EC 6.3.2.5)	-2.77	1.17E-03
7	741078	742079	+	Coenzyme F420-dependent N52CN10-methylene tetrahydromethanopterin reductase and related flavin-dependent oxidoreductases	-2.88	2.96E-31
7	742135	742581	+	Deoxyuridine 5'-triphosphate nucleotidohydrolase (EC 3.6.1.23)	-4.22	9.76E-20
7	742616	742933	-	Phenylalanine-specific permease	-3.25	1.26E-03
7	75623	76495	-	Pirin-related protein	2.37	2.03E-06
7	744152	746449	-	ATP-dependent Clp protease ATP-binding subunit ClpA	2.71	0.00E+00
7	746446	746760	-	ATP-dependent Clp protease adaptor protein ClpS	2.93	0.00E+00
7	753284	754267	-	Fumarylacetoacetate hydrolase family protein	2.46	2.68E-05
7	76621	77661	+	Transcriptional regulator2C LysR family	2.93	4.86E-15
7	757935	759185	-	Transcriptional regulator2C LysR family	4.35	6.47E-07
7	759315	760286	-	Outer membrane protein (porin)	10.69	1.16E-08
7	762403	763956	-	Inner membrane component of tripartite multidrug resistance system	2.07	1.10E-05

7	764614	765048	-	Transcriptional regulator2C MarR family	2.19	2.29E-11
7	765520	765789	-	Mobile element protein	2.17	1.59E-06
7	767523	768617	+	Cysteine desulfurase (EC 2.8.1.7)	12.13	0.00E+00
7	768897	771605	+	Membrane alanine aminopeptidase N (EC 3.4.11.2)	5.36	5.68E-11
7	771665	773461	+	Xaa-Pro aminopeptidase (EC 3.4.11.9)	3.53	6.02E-14
7	78081	78494	-	Biopolymer transport protein ExbD/TolR	2.93	2.90E-05
7	773931	775286	-	two-component sensor kinase	3.22	0.00E+00
7	776300	776752	+	Transcriptional regulator2C AsnC family	5.66	0.00E+00
7	777129	779444	+	Ferrichrome-iron receptor	6.38	0.00E+00
7	779543	781267	+	ABC transporter2C ATP-binding protein	2.29	4.59E-07
7	789041	789295	-	Mobile element protein	4.84	5.14E-05
7	789875	790399	-	Mobile element protein	3.11	2.93E-09
7	790797	791141	-	Mobile element protein	8.33	1.10E-03
7	792246	792929	+	Mobile element protein	17.29	0.00E+00
7	793098	793256	+	hypothetical protein	4.11	6.86E-04
7	795825	796988	-	Salicylate hydroxylase (EC 1.14.13.1)	2.59	2.52E-09
7	797241	797762	-	Lactoylglutathione lyase and related lyases	3.34	2.52E-04
7	797929	798606	-	3-isopropylmalate dehydratase small subunit (EC 4.2.1.33)	8.89	7.52E-04
7	798603	800021	-	3-isopropylmalate dehydratase large subunit (EC 4.2.1.33)	10.13	7.15E-11
7	800018	801010	-	Hydroxymethylglutaryl-CoA lyase (EC 4.1.3.4)	29.28	0.00E+00
7	801025	802239	-	L-carnitine dehydratase/bile acid-inducible protein F (EC 2.8.3.16)	10.14	0.00E+00
7	802274	803431	-	hypothetical protein	7.63	6.88E-09
7	803826	805508	+	22C3-dihydroxybenzoate-AMP ligase (EC 2.7.7.58)	8.24	4.87E-04
7	806576	807331	+	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)	5.36	1.94E-08
7	807347	808657	+	p-cumate dioxygenase large subunit (CmtAb)	5.58	1.17E-14
7	808668	809174	+	p-cumate dioxygenase small subunit (CmtAc)	3.73	8.12E-06
7	811256	812917	+	Salicylate hydroxylase (EC 1.14.13.1)	8.99	0.00E+00
7	813170	814162	+	4-hydroxy-tetrahydrodipicolinate synthase (EC 4.3.3.7)	2.48	7.70E-04
7	814198	815061	+	Beta-ketoadipate enol-lactone hydrolase (EC 3.1.1.24)	2.41	2.48E-15
7	816011	817528	+	Aldehyde dehydrogenase (EC 1.2.1.3)	4.08	0.00E+00
7	817550	819139	+	Phytoene dehydrogenase and related proteins	2.62	0.00E+00
7	819884	821068	+	3-ketoacyl-CoA thiolase (EC 2.3.1.16) @ Acetyl-CoA acetyltransferase (EC 2.3.1.9)	3.20	8.22E-07
7	821087	821767	-	hypothetical protein	3.72	2.93E-08
7	822251	822460	-	Mobile element protein	14.09	0.00E+00
7	822520	822924	-	Mobile element protein	3.37	5.73E-04
7	80396	80647	-	Bacterioferritin-associated ferredoxin	-11.01	1.84E-14
7	823164	823280	-	FIG00460579: hypothetical protein	5.05	0.00E+00
7	825478	825873	-	hypothetical protein	2.77	1.84E-14
7	826562	826762	-	Mobile element protein	5.67	9.67E-09
7	827920	828366	+	Putative phage-encoded peptidoglycan binding protein	8.71	9.24E-14
7	829511	830347	+	hypothetical protein	2.72	4.84E-03
7	832924	833985	-	Phage integrase	2.14	6.97E-09
7	834300	835556	-	Isocitrate dehydrogenase [NADP] (EC 1.1.1.42)	-2.21	8.52E-93
7	838255	840360	+	Translation elongation factor G	2.30	0.00E+00

7	840390	840524	+	hypothetical protein	3.28	0.00E+00
7	840521	841039	+	Predicted mannose-6-phosphate isomerase	2.71	0.00E+00
7	841259	842914	+	ABC-type dipeptide transport system2C periplasmic component	2.11	8.52E-10
7	847484	848353	+	Glycine-rich cell wall structural protein precursor	7.36	0.00E+00
7	848534	849139	+	FIG00459032: hypothetical protein	3.81	0.00E+00
7	849269	850114	-	Oxidoreductase2C aldo/keto reductase family	6.27	0.00E+00
7	852703	853959	+	FIG00453118: hypothetical protein	6.21	1.03E-04
7	82568	84091	-	Fumarate hydratase class I2C aerobic (EC 4.2.1.2)	-2.48	3.26E-24
7	853984	854337	-	FIG00453813: hypothetical protein	4.94	7.77E-03
7	854463	855878	+	Transcriptional regulator2C GntR family domain / Aspartate aminotransferase (EC 2.6.1.1)	3.03	0.00E+00
7	857772	859052	+	FOG: EAL domain	2.68	4.94E-14
7	859087	860154	-	Putative RNA polymerase sigma factor	2.01	3.86E-06
7	869291	870841	-	Proposed peptidoglycan lipid II flippase MurJ	2.75	2.57E-09
7	85067	85966	-	Permease of the drug/metabolite transporter (DMT) superfamily	2.00	1.18E-04
7	876260	876751	-	Transcriptional regulator	2.37	2.73E-03
7	86387	88333	+	Acetyl-coenzyme A synthetase (EC 6.2.1.1)	2.16	5.37E-07
7	886183	887031	+	FIG000875: Thioredoxin domain-containing protein EC-YbbN	6.94	1.13E-05
7	888270	888908	+	Pyridoxamine 5'-phosphate oxidase (EC 1.4.3.5)	2.24	0.00E+00
7	889067	890281	+	Cyclopropane-fatty-acyl-phospholipid synthase (EC 2.1.1.79)	2.25	0.00E+00
7	890370	891623	+	FIG003003: hypothetical protein	2.53	7.16E-15
7	891692	892240	-	Peptide methionine sulfoxide reductase MsrA (EC 1.8.4.11)	2.83	9.09E-10
7	893005	893511	-	Leucine-responsive regulatory protein2C regulator for leucine (or lrp) regulon and high-affinity branched-chain amino acid transport system	2.71	0.00E+00
7	893672	894313	+	Kynurenine formamidase2C bacterial (EC 3.5.1.9)	13.58	0.00E+00
7	894396	895646	+	Kynureninase (EC 3.7.1.3)	15.39	0.00E+00
7	88508	88816	+	FIG152265: Sodium:solute symporter associated protein	7.97	4.61E-03
7	895657	896640	+	Tryptophan 22C3-dioxygenase (EC 1.13.11.11)	6.05	0.00E+00
7	902761	903441	+	Deoxyadenosine kinase (EC 2.7.1.76) / Deoxyguanosine kinase (EC 2.7.1.113)	-2.23	3.73E-14
7	904420	906309	-	Para-aminobenzoate synthase2C aminase component (EC 2.6.1.85) / Aminodeoxychorismate lyase (EC 4.1.3.38)	2.04	1.78E-09
7	88813	90831	+	Acetate permease ActP (cation/acetate symporter)	2.31	0.00E+00
7	906334	907467	-	Chaperone protein DnaJ	4.62	2.14E-09
7	907513	907785	+	hypothetical protein	4.71	2.52E-08
7	907763	909709	-	Chaperone protein DnaK	6.83	1.34E-11
7	910299	910892	-	Heat shock protein GrpE	5.12	1.11E-13
7	913081	914100	-	Heat-inducible transcription repressor HrcA	2.03	1.10E-06
7	928620	928934	+	Phage-related protein	2.83	1.90E-07
7	928935	929282	+	transcriptional regulator2C XRE family	5.81	0.00E+00
7	929326	930531	-	Flavohemoprotein (Hemoglobin-like protein) (Flavohemoglobin) (Nitric oxide dioxygenase) (EC 1.14.12.17)	15.91	0.00E+00
7	931440	932864	+	D-Lactate dehydrogenase2C cytochrome c-dependent (EC 1.1.2.4)	2.48	9.39E-12
7	932923	934416	+	Glycolate dehydrogenase (EC 1.1.99.14)2C subunit GlcD	2.56	5.37E-14
7	91680	91826	-	Mycobacteriophage Barnyard protein gp56	2.71	2.37E-03
7	942069	942245	-	hypothetical protein	2.13	8.90E-04
7	947337	948527	+	tRNA-guanine transglycosylase (EC 2.4.2.29)	-2.38	6.82E-05

7	92308	94839	+	PUTATIVE VGR-RELATED PROTEIN	-4.15	3.19E-07
7	949257	951317	+	Protein-export membrane protein SecD (TC 3.A.5.1.1)	-2.42	3.16E-05
7	951333	952283	+	Protein-export membrane protein SecF (TC 3.A.5.1.1)	-3.92	4.17E-07
7	952459	953742	-	Citrate-proton symporter	-3.56	1.80E-06
7	953929	954516	-	Protein yceI precursor	-3.20	8.27E-03
7	954581	955168	-	Probable signal peptide protein	-2.14	3.21E-05
7	957756	959441	+	Paraquat-inducible protein B	-2.03	9.74E-17
7	95370	95846	+	hypothetical protein	-6.86	4.56E-68
7	96103	98736	+	hypothetical protein	-2.91	2.55E-21
7	969026	970639	+	Ferredoxin-dependent glutamate synthase (EC 1.4.7.1)	-2.26	7.44E-08
7	970819	971652	-	ABC-type nitrate/sulfonate/bicarbonate transport system2C permease component	-3.23	2.27E-03
7	971701	972576	-	ABC-type nitrate/sulfonate/bicarbonate transport system2C ATPase component	-4.42	4.12E-05
7	981193	982758	+	IMP cyclohydrolase (EC 3.5.4.10) / Phosphoribosylaminoimidazolecarboxamide formyltransferase (EC 2.1.2.3)	-2.16	2.26E-11
7	984283	985347	+	Holliday junction DNA helicase RuvB	2.05	0.00E+00
7	985386	986345	+	FIG00953497: hypothetical protein	2.38	1.26E-05
7	990961	991353	-	SSU ribosomal protein S9p (S16e)	-2.09	3.85E-23
7	101691	111191	+	Putative large exoprotein involved in heme utilization or adhesion of ShlA/HecA/FhaA family	-2.96	1.18E-26
7	997505	998185	-	Glutamate Aspartate transport system permease protein GltK (TC 3.A.1.3.4)	-2.35	9.60E-03
7	111909	113681	+	Channel-forming transporter/cytolysins activator of TpsB family	-3.30	1.19E-22
7	113771	115375	+	FOG: TPR repeat	-5.19	1.07E-12
7	115429	116604	+	Similarity with glutathionylspermidine synthase (EC 6.3.1.8)2C group 1	-8.37	1.25E-06
7	1041160	1042176	-	L-arabinose transport system permease protein (TC 3.A.1.2.2)	-2.24	9.16E-05
7	1043900	1044898	-	L-arabinose-binding periplasmic protein precursor AraF (TC 3.A.1.2.2)	-2.29	3.10E-04
7	1062437	1062937	+	Thiol peroxidase2C Tpx-type (EC 1.11.1.15)	-2.68	3.04E-05
7	122050	123285	-	Citrate synthase (si) (EC 2.3.3.1)	3.58	1.28E-12
7	1071010	1071630	-	N-acetylmuramoyl-L-alanine amidase (EC 3.5.1.28) AmpD	2.32	3.55E-06
7	1071627	1072019	-	Pyrimidine deaminase	3.15	3.47E-06
7	1072025	1072954	-	FIG001154: CcsA-related protein	2.27	5.30E-04
7	1085383	1086648	+	Type IV fimbrial assembly2C ATPase PilB	2.56	4.07E-03
7	1086645	1087871	+	Type IV fimbrial assembly protein PilC	2.16	1.89E-03
7	831130	832971	+	Fibronectin type III domain protein	2.78	2.06E-14
7	983549	984130	+	Holliday junction DNA helicase RuvA	5.19	0.00E+00
8	1002	2192	+	Translation elongation factor Tu	-2.68	4.27E-66
8	3462	3893	+	LSU ribosomal protein L11p (L12e)	-2.22	5.58E-07
8	3894	4592	+	LSU ribosomal protein L1p (L10Ae)	-2.56	0.00E+00
8	4895	5392	+	LSU ribosomal protein L10p (P0)	-2.86	3.60E-04
8	5465	5842	+	LSU ribosomal protein L7/L12 (P1/P2)	-3.89	5.11E-30
8	6223	10329	+	DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	-3.66	2.61E-102
8	10351	14592	+	DNA-directed RNA polymerase beta' subunit (EC 2.7.7.6)	-4.49	3.87E-11
8	17470	17940	+	SSU ribosomal protein S7p (S5e)	-2.22	1.13E-03
8	18080	20182	+	Translation elongation factor G	-3.18	9.37E-09
8	21551	21862	+	SSU ribosomal protein S10p (S20e)	-2.29	9.21E-129

8	23359	23673	+	LSU ribosomal protein L23p (L23Ae)	-2.73	3.98E-15
8	23676	24503	+	LSU ribosomal protein L2p (L8e)	-2.66	5.27E-20
8	24513	24788	+	SSU ribosomal protein S19p (S15e)	-3.43	1.91E-07
8	24801	25130	+	LSU ribosomal protein L22p (L17e)	-4.21	6.35E-12
8	25175	25942	+	SSU ribosomal protein S3p (S3e)	-3.25	1.03E-03
8	25945	26361	+	LSU ribosomal protein L16p (L10e)	-3.44	9.16E-05
8	26372	26566	+	LSU ribosomal protein L29p (L35e)	-3.93	2.13E-08
8	26563	26835	+	SSU ribosomal protein S17p (S11e)	-3.70	1.16E-57
8	27847	28386	+	LSU ribosomal protein L5p (L11e)	-2.43	7.89E-19
8	28394	28699	+	SSU ribosomal protein S14p (S29e) @ SSU ribosomal protein S14p (S29e)2C zinc-independent	-2.24	1.21E-05
8	28714	29109	+	SSU ribosomal protein S8p (S15Ae)	-2.94	8.60E-15
8	29128	29658	+	LSU ribosomal protein L6p (L9e)	-2.81	4.22E-19
8	29671	30036	+	LSU ribosomal protein L18p (L5e)	-2.83	3.55E-05
8	30051	30569	+	SSU ribosomal protein S5p (S2e)	-2.21	1.14E-05
8	30580	30762	+	LSU ribosomal protein L30p (L7e)	-3.01	9.89E-04
8	31275	32621	+	Preprotein translocase secY subunit (TC 3.A.5.1.1)	-2.53	1.94E-47
8	32629	32847	+	Translation initiation factor 1	-2.36	9.29E-10
8	34728	35705	+	DNA-directed RNA polymerase alpha subunit (EC 2.7.7.6)	-3.38	6.66E-04
8	49350	51692	-	Multimodular transpeptidase-transglycosylase (EC 2.4.1.129) (EC 3.4.-.-)	-2.16	1.75E-13
8	60374	60751	-	Permeases of the major facilitator superfamily	2.18	5.40E-06
8	67839	69305	+	Glutamate synthase [NADPH] small chain (EC 1.4.1.13)	-2.61	5.49E-16
8	72459	73589	+	Glycine oxidase ThiO (EC 1.4.3.19)	2.56	0.00E+00
8	76730	77497	+	Uncharacterized ABC transporter2C permease component YrbE	2.09	1.97E-03
8	78148	79110	+	Nucleoside ABC transporter2C periplasmic nucleoside-binding protein	-2.35	1.90E-19
8	81278	82033	+	ABC-type multidrug transport system2C permease component	-2.63	4.92E-19
8	82049	82288	+	YrbA protein	-4.04	1.09E-10
8	82301	83662	+	UDP-N-acetylglucosamine 1-carboxyvinyltransferase (EC 2.5.1.7)	-2.24	1.35E-08
8	83659	84357	+	ATP phosphoribosyltransferase (EC 2.4.2.17)	-2.69	1.36E-12
8	84385	85704	+	Histidinol dehydrogenase (EC 1.1.1.23)	-3.38	7.55E-87
8	89586	90359	+	Imidazole glycerol phosphate synthase cyclase subunit (EC 4.1.3.-)	-2.08	1.26E-04
8	90361	90786	+	Phosphoribosyl-AMP cyclohydrolase (EC 3.5.4.19)	-3.39	4.38E-09
8	90783	91148	+	Phosphoribosyl-ATP pyrophosphatase (EC 3.6.1.31)	-3.06	2.52E-03
8	91745	92113	+	FIG146285: Diadenosine tetraphosphate (Ap4A) hydrolase and other HIT family hydrolases	2.66	3.52E-05
8	118798	119691	+	Transcriptional regulator of various polyols utilization2C AraC family	-2.43	5.55E-03
8	121373	122746	+	Lysophospholipase (EC 3.1.1.5)	4.88	3.92E-11
8	131127	131852	+	Predicted regulator PutR for proline utilization2C GntR family	5.39	8.83E-11
8	131899	132705	+	D-beta-hydroxybutyrate dehydrogenase (EC 1.1.1.30)	2.75	1.08E-04
8	132726	133658	+	FIG00455516: hypothetical protein	2.26	2.48E-03
8	153668	153970	+	hypothetical protein	-2.19	5.74E-03
8	154059	155186	+	FIG00454994: hypothetical protein	-2.12	3.75E-07
8	155876	156142	+	hypothetical protein	4.75	1.40E-14
8	180043	180555	+	Transcriptional regulator2C TetR family	-3.48	2.68E-31
8	180574	181365	-	Probable transmembrane protein	-3.88	6.53E-12

8	181362	182708	-	Uncharacterized protein ImpJ/VasE	-3.45	1.28E-13
8	182800	183414	-	Type VI secretion lipoprotein/VasD	-3.78	2.86E-08
8	183812	184444	+	FOG: TPR repeat	-3.41	1.38E-03
8	184487	185026	+	Uncharacterized protein ImpB	-3.69	7.22E-75
8	185057	186550	+	Uncharacterized protein ImpC	-4.74	1.93E-187
8	186626	187129	+	Uncharacterized protein ImpD	-9.32	1.24E-06
8	187200	187679	+	Uncharacterized protein ImpF	-5.44	2.05E-12
8	187740	189581	+	Protein ImpG/VasA	-4.83	2.74E-42
8	189545	190639	+	Uncharacterized protein ImpH/VasB	-9.31	5.76E-151
8	190688	193369	+	ClpB protein	-9.04	7.52E-228
8	193373	194524	+	Uncharacterized protein ImpA	-7.53	3.37E-180
8	194622	195611	-	Outer membrane protein assembly factor YaeT precursor	-3.90	1.22E-13
8	195616	196608	-	FIG00977343: hypothetical protein	-2.58	3.41E-09
8	196605	200528	-	IcmF-related protein	-2.86	2.26E-42
8	214425	215249	+	Lysine-arginine-ornithine-binding periplasmic protein precursor (TC 3.A.1.3.1)	3.84	0.00E+00
8	218736	220208	-	D-mannonate oxidoreductase (EC 1.1.1.57)	4.49	0.00E+00
8	225600	225833	-	hypothetical protein	2.10	3.73E-04
8	242752	244248	+	Acetate permease ActP (cation/acetate symporter)	-2.38	3.12E-05
8	244372	245364	-	ortholog of Bordetella pertussis (BX470248) BP3300	-4.08	2.20E-03
8	245416	246897	-	Succinate-semialdehyde dehydrogenase [NAD(P)+] (EC 1.2.1.16)	-2.23	1.77E-08
8	271325	271528	-	hypothetical protein	-2.08	4.19E-03
8	272659	274053	+	Signal transduction histidine kinase	26.67	0.00E+00
8	274107	274325	+	hypothetical protein	38.39	6.98E-08
8	274342	274473	-	hypothetical protein	54.43	0.00E+00
8	274623	275627	-	HEAT repeat-containing protein	4.88	6.18E-12
8	275654	275908	-	Adenylylsulfate reductase beta-subunit (EC 1.8.99.2)	15.08	2.84E-03
8	281008	281343	+	Transcriptional regulator2C ArsR family	2.80	2.31E-09
8	282731	284056	+	TRAP-type uncharacterized transport system2C periplasmic component	3.28	1.20E-05
8	284194	284502	+	Phosphatidylserine/phosphatidylglycerophosphate/cardiolipi n synthases and related enzymes	2.90	1.40E-14
8	284543	285991	-	Methylamine utilization protein mauG precursor	4.96	6.48E-04
8	287907	288065	+	hypothetical protein	12.12	2.45E-05
8	300836	303058	+	Ferrichrome-iron receptor	-5.31	1.17E-15
8	304741	305139	-	Pyruvate/2-oxoglutarate dehydrogenase complex2C dihydrolipoamide dehydrogenase (E3) component2C and related enzymes	2.80	8.22E-07
8	318259	319413	-	Outer membrane protein (porin)	6.75	0.00E+00
8	337319	338380	-	Probable serine protease do-like precursor (EC 3.4.21.-)	32.81	1.62E-14
8	339749	341077	-	Heavy-chain fibroin (Fragment)	-2.33	2.69E-07
8	343944	344219	-	hypothetical protein	7.46	5.49E-05
8	348951	350552	+	ABC-type dipeptide transport system2C periplasmic component	9.44	0.00E+00
8	350600	351676	+	Dipeptide transport system permease protein DppB (TC 3.A.1.5.2)	2.89	1.32E-04
8	352723	353649	+	Dipeptide transport ATP-binding protein DppD (TC 3.A.1.5.2)	4.51	4.46E-03
8	353646	354428	+	Dipeptide transport ATP-binding protein DppF (TC 3.A.1.5.2)	11.11	1.62E-07
8	354579	355202	-	L-lysine permease	2.53	2.35E-04

8	355389	355997	+	Cupin domain-containing protein	3.70	5.63E-12
8	361518	363068	-	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)	3.50	9.09E-08
8	363281	363529	-	hypothetical protein	3.02	0.00E+00
8	363534	363686	-	hypothetical protein	3.96	0.00E+00
8	363680	364402	-	No significant database matches	5.46	0.00E+00
8	366425	367810	-	Short-chain alcohol dehydrogenase family	-2.21	1.68E-13
8	377821	378135	+	hypothetical protein	3.24	1.10E-03
8	378434	378748	+	hypothetical protein	5.99	2.80E-03
8	381328	381825	-	Rhodanese-related sulfurtransferase	4.19	6.20E-04
8	383597	383797	-	hypothetical protein	3.36	5.92E-04
8	384122	384310	+	hypothetical protein	2.15	7.02E-05
8	385721	386512	-	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)	3.89	2.40E-05
8	386702	387904	+	Polyhydroxyalkanoic acid synthase	9.39	7.23E-07
8	387896	388693	-	hypothetical protein	9.42	0.00E+00
8	414261	415193	+	Transcriptional regulator2C LysR family	2.56	1.68E-05
8	415273	415884	-	LysE family protein	2.71	4.53E-04
8	416132	416344	-	SSU ribosomal protein S21p	5.22	0.00E+00
8	416846	417049	+	Cold shock protein CspA	3.55	0.00E+00
8	417320	417454	+	hypothetical protein	16.62	8.25E-09
8	417816	417971	+	hypothetical protein	3.29	0.00E+00
8	418386	418652	-	Translation initiation factor 1	4.83	0.00E+00
8	419192	420256	-	N-ethylmaleimide reductase (EC 1.-.-.-)	-2.21	4.61E-03
8	420423	420749	-	Transcriptional regulator2C ArsR family	-2.41	1.33E-03
8	439670	440992	+	FIG00460817: hypothetical protein	2.45	1.30E-04
8	442732	443532	+	Amino acid ABC transporter2C ATP-binding protein	2.20	1.90E-06
8	444910	445623	+	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)	3.15	7.01E-03
8	446095	447600	-	Outer membrane component of tripartite multidrug resistance system	4.82	4.17E-08
8	447741	448304	+	Transcriptional regulator2C TetR family	15.68	0.00E+00
8	448382	449512	+	Probable Co/Zn/Cd efflux system membrane fusion protein	6.16	1.15E-04
8	449509	452607	+	Cation efflux system protein	3.27	3.81E-05
8	452671	453108	-	Protocatechuate 42C5-dioxygenase beta chain (EC 1.13.11.8)	3.17	2.26E-03
8	459856	459981	-	hypothetical protein	4.08	1.39E-07
8	465234	466211	-	ABC transporter substrate-binding protein	2.34	6.04E-04
8	479176	480075	+	Metallo-beta-lactamase superfamily protein PA0057	2.41	1.31E-13
8	507373	507537	+	hypothetical protein	-13.36	3.30E-05
8	513696	514049	+	DNA-invertase	6.39	0.00E+00
8	515288	515593	+	DNA-binding response regulator	2.59	0.00E+00
8	515691	516833	-	Hydroxymethylglutaryl-CoA reductase (EC 1.1.1.34)	3.17	1.91E-10
8	517363	518385	-	3-oxoacyl-[acyl-carrier-protein] synthase2C KASIII (EC 2.3.1.180)	3.83	2.94E-10
8	518845	522735	-	Malonyl CoA-acyl carrier protein transacylase (EC 2.3.1.39)	2.70	1.36E-04
8	522812	524089	-	Alcohol dehydrogenase (EC 1.1.1.1)	3.54	6.74E-08
8	524207	524965	-	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)	5.60	1.40E-14
8	525463	526626	+	4-carboxymuconolactone decarboxylase (EC 4.1.1.44)	6.58	0.00E+00
8	526749	527234	+	MaoC family protein	6.37	1.49E-04

8	527573	528337	-	hypothetical protein	4.11	0.00E+00
8	533780	534541	+	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)	2.72	1.01E-03
8	558376	559914	+	Amino acid transporters	6.10	3.46E-09
8	561103	561981	+	Putative DNA-binding protein in cluster with Type I restriction-modification system	2.40	3.23E-03
8	563960	564889	+	Muramoyltetrapeptide carboxypeptidase (EC 3.4.17.13)	8.14	0.00E+00
8	565039	565296	-	hypothetical protein	7.66	0.00E+00
8	568710	569660	+	Uricase (urate oxidase) (EC 1.7.3.3)	4.00	0.00E+00
8	569657	570193	+	Urate oxidase (EC 1.7.3.3)	3.03	2.39E-07
8	80355	81281	+	ABC-type multidrug transport system2C ATPase component	-2.02	6.24E-11
9	1055	2089	-	S-adenosylmethionine:tRNA ribosyltransferase-isomerase (EC 5.-.-.-)	2.59	1.57E-04
9	2987	3556	+	Transcriptional regulator	11.65	1.80E-12
9	3875	4048	-	FIG00453086: hypothetical protein	8.60	1.46E-07
9	7513	8244	-	Membrane proteins related to metalloendopeptidases	2.19	1.75E-06
9	11005	11913	-	Transcriptional regulator	10.43	0.00E+00
9	13641	15776	+	Methyl-accepting chemotaxis protein	-2.71	9.91E-03
9	15773	16429	+	Chemotaxis motB protein	-2.79	7.57E-10
9	17511	17753	+	hypothetical protein	74.34	0.00E+00
9	17961	19622	-	RNA polymerase sigma factor RpoD	107.24	0.00E+00
9	19593	19820	+	hypothetical protein	113.79	3.35E-07
9	19784	20218	-	hypothetical protein	45.05	0.00E+00
9	21447	22067	+	hypothetical protein	5.09	4.93E-03
9	29667	31046	+	Cytochrome c peroxidase	2.31	7.91E-04
9	36223	38847	+	Alanyl-tRNA synthetase (EC 6.1.1.7)	-2.00	6.51E-27
9	39746	39913	-	hypothetical protein	-3.25	8.33E-04
9	39917	41065	+	Fe-containing alcohol dehydrogenase	-2.18	1.06E-03
9	41089	41586	+	putative 4-hydroxybenzoyl-CoA thioesterase	-3.26	9.49E-03
9	45573	45734	+	hypothetical protein	2.29	1.73E-06
9	53117	54448	-	Protein hipA	-3.67	1.05E-08
9	54965	55774	-	5-deoxy-glucuronate isomerase (EC 5.3.1.-)	-10.24	3.29E-92
9	55771	56685	-	Inosose dehydratase (EC 4.2.1.44)	-10.36	1.10E-03
9	56710	58644	-	Epi-inositol hydrolase (EC 3.7.1.-)	-10.92	7.42E-57
9	58641	60674	-	5-keto-2-deoxygluconokinase (EC 2.7.1.92) / uncharacterized domain	-13.63	1.80E-16
9	60765	61580	-	Inositol transport system ATP-binding protein	-10.88	1.53E-14
9	61609	62772	-	Inositol transport system permease protein	-8.17	4.12E-35
9	62917	63975	-	Inositol transport system sugar-binding protein	-6.78	8.56E-19
9	64211	65110	+	Predicted transcriptional regulator of the myo-inositol catabolic operon	-8.56	2.79E-20
9	65103	66116	+	Myo-inositol 2-dehydrogenase 1 (EC 1.1.1.18)	-6.98	9.22E-25
9	66157	67206	+	Myo-inositol 2-dehydrogenase (EC 1.1.1.18)	-8.61	1.27E-16
9	72944	73258	-	hypothetical protein	7.00	7.12E-03
9	77524	80403	-	Valyl-tRNA synthetase (EC 6.1.1.9)	-2.10	1.92E-09
9	85549	87453	-	Propionate--CoA ligase (EC 6.2.1.17)	2.18	4.84E-03
9	87430	87633	+	hypothetical protein	6.07	2.25E-07
9	87810	88688	-	Membrane fusion component of tripartite multidrug resistance system	-2.20	2.01E-07

9	98014	99333	+	Ribonuclease BN (EC 3.1.-.-)	3.34	0.00E+00
9	99341	99667	-	FIG027190: Putative transmembrane protein	5.54	0.00E+00
9	110415	111119	+	Succinyl-CoA:3-ketoacid-coenzyme A transferase subunit A (EC 2.8.3.5)	2.85	2.24E-10
9	111122	111763	+	Succinyl-CoA:3-ketoacid-coenzyme A transferase subunit B (EC 2.8.3.5)	2.41	3.75E-03
9	112095	112970	+	probable hydrolase	3.26	2.85E-12
9	113424	113546	-	hypothetical protein	2.55	1.49E-07
9	123989	124363	+	Transcriptional regulator2C MerR family	-2.48	4.55E-08
9	125230	126081	-	Transcriptional regulator2C LysR family	3.76	0.00E+00
9	128211	128570	+	hypothetical protein	5.75	0.00E+00
9	132096	133271	+	Outer membrane protein (porin)	4.16	2.14E-04
9	133307	134746	+	MmgE/PrpD family protein	2.65	5.81E-03
9	135958	136791	+	COGs COG3777	4.53	8.56E-05
9	136863	138245	+	Putative metabolite transport protein	2.08	3.03E-04
9	157649	158893	+	D-amino acid dehydrogenase small subunit (EC 1.4.99.1)	3.36	8.33E-03
9	160648	161532	+	dTDP-rhamnosyl transferase RfbF (EC 2.-.-.-)	2.67	9.71E-03
9	161634	162569	+	L-2-keto-3-deoxyarabonate dehydratase (EC 4.2.1.43)	3.87	4.99E-05
9	178141	179418	+	Opine oxidase subunit B	2.70	9.99E-04
9	179841	180872	+	Aldehyde dehydrogenase (EC 1.2.1.3)	2.79	2.33E-04
9	185372	185848	-	hypothetical protein	-2.73	3.37E-07
9	186578	188521	-	Two-component response regulator	3.02	1.80E-07
9	188932	189204	+	hypothetical protein	2.82	1.57E-08
9	189218	189427	-	hypothetical protein	3.06	1.81E-03
9	190040	190498	+	hypothetical protein	2.56	2.45E-04
9	193965	194162	-	FIG00453722: hypothetical protein	2.20	0.00E+00
9	194690	197041	-	DinG family ATP-dependent helicase CPE1197	7.71	0.00E+00
9	197038	198762	-	Hypothetical protein2C restriction endonuclease-like VRR-NUC domain	7.14	0.00E+00
9	202476	202724	-	hypothetical protein	2.71	3.12E-05
9	206205	207803	-	L-lactate permease	3.03	3.50E-11
9	208028	208468	-	glycine-rich protein	10.27	2.48E-15
9	208516	208689	+	hypothetical protein	12.20	4.86E-15
9	209329	211260	-	Hydroxymethylpyrimidine phosphate synthase ThiC (EC 4.1.99.17)	-2.92	2.54E-65
9	211604	212014	-	Regulator of nucleoside diphosphate kinase	2.85	2.26E-11
9	212508	213341	+	FIG00453123: hypothetical protein	3.34	2.03E-13
9	216545	217444	+	MotA/TolQ/ExbB proton channel family protein	-2.08	3.86E-08
9	217451	217873	+	Biopolymer transport protein ExbD/TolR	-2.64	1.07E-05
9	220868	223567	+	Outer membrane receptor proteins2C mostly Fe transport	-3.06	1.04E-15
9	223648	225546	+	ABC transporter2C periplasmic substrate-binding protein	-2.61	7.56E-03
9	225555	226604	+	Oligopeptide transport system permease protein OppB (TC 3.A.1.5.1)	-2.61	2.72E-03
9	229343	230779	+	TldD family protein2C Beta/Gamma-proteobacterial subgroup	-2.64	8.01E-04
9	230779	232140	+	TldE/PmbA family protein2C Beta/Gamma-proteobacterial subgroup	-3.23	2.01E-09
9	232810	233415	+	Histone acetyltransferase HPA2 and related acetyltransferases	-4.17	8.26E-03
9	241018	241179	+	putative membrane protein	-8.25	3.25E-04
9	241226	241768	+	Bacterioferritin	-8.62	1.68E-17

9	241987	243246	-	Permeases of the major facilitator superfamily	-2.86	4.51E-06
9	249016	250074	-	Catabolite control protein A	2.63	5.57E-03
9	262781	263398	-	Glutathione S-transferase (EC 2.5.1.18)	-2.16	1.77E-07
9	276085	277695	+	sensory box histidine kinase/response regulator	22.37	1.90E-03
9	277692	281273	+	sensory box histidine kinase/response regulator	5.07	4.21E-04
9	281302	282513	+	diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)	4.22	3.06E-04
9	296313	298349	+	Transport ATP-binding protein CydCD	-2.03	9.30E-04
9	299756	301180	+	PF00070 family2C FAD-dependent NAD(P)-disulphide oxidoreductase	2.09	2.11E-05
9	302605	303099	+	phosphoesterase2C putative	2.09	1.22E-04
9	305017	305175	+	hypothetical protein	2.99	3.73E-10
9	305168	305533	-	hypothetical protein	6.16	0.00E+00
9	318425	318598	-	hypothetical protein	2.81	1.10E-04
9	318560	319702	+	Nitrogen regulation protein NtrB (EC 2.7.13.3)	4.26	1.32E-08
9	319736	321250	+	Nitrogen regulation protein NR(I)	3.64	7.07E-06
9	332681	334783	-	Oligopeptidase A (EC 3.4.24.70)	2.87	0.00E+00
9	341207	343912	+	Pyruvate dehydrogenase E1 component (EC 1.2.4.1)	-2.95	2.06E-77
9	344007	345683	+	Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex (EC 2.3.1.12)	-3.31	6.31E-10
9	345723	345932	+	hypothetical protein	-4.80	7.06E-06
9	354520	354933	+	Low molecular weight protein tyrosine phosphatase (EC 3.1.3.48)	2.28	1.29E-03
9	361542	362279	-	Outer membrane lipoprotein	3.66	0.00E+00
9	380422	381396	-	33 kDa chaperonin (Heat shock protein 33) (HSP33)	2.56	2.08E-04
9	381508	382032	-	carbonic anhydrase2C family 3	2.26	5.80E-10
9	383387	384181	+	Hydrolase2C alpha/beta fold family functionally coupled to Phosphoribulokinase	4.14	0.00E+00
9	385328	385963	+	Hypothetical YciO protein2C TsaC/YrdC paralog	2.29	1.08E-03
9	397077	398417	+	Histidyl-tRNA synthetase (EC 6.1.1.21)	-2.88	4.54E-206
9	400715	402016	+	GTP-binding protein EngA	-2.01	1.65E-29
9	410075	411961	+	Kup system potassium uptake protein	-2.01	7.02E-07
9	412119	414464	-	Transcription accessory protein (S1 RNA-binding domain)	-2.59	4.54E-99
9	420111	420650	+	FKBP-type peptidyl-prolyl cis-trans isomerase SlyD (EC 5.2.1.8)	2.04	0.00E+00
9	420712	421767	-	Probable transmembrane protein	2.89	0.00E+00
9	421979	424699	-	DNA mismatch repair protein MutS	-2.00	2.64E-09
9	427791	428657	+	tRNA:Cm32/Um32 methyltransferase	-2.52	5.12E-05
9	430714	431208	-	Peptidyl-prolyl cis-trans isomerase PpiB (EC 5.2.1.8)	-2.43	2.60E-10
9	438600	439850	+	Aspartokinase (EC 2.7.2.4)	-2.42	8.86E-04
9	442599	443393	+	FIG018329: 1-acyl-sn-glycerol-3-phosphate acyltransferase	-2.74	3.56E-07
9	443368	443667	+	Acyl carrier protein (ACP1)	-2.81	1.57E-06
9	444620	446344	+	FIGfam138462: Acyl-CoA synthetase2C AMP-(fatty) acid ligase / (3R)-hydroxymyristoyl-[ACP] dehydratase (EC 4.2.1.-)	-2.39	1.13E-13
9	446341	448056	+	FIG143263: Glycosyl transferase / Lysophospholipid acyltransferase	-2.35	3.17E-05
9	448043	449635	+	Putative histidine ammonia-lyase protein	-3.10	1.55E-17
9	450051	450758	+	FIG027190: Putative transmembrane protein	-3.10	3.92E-04
9	450758	453178	+	FIG021862: membrane protein2C exporter	-6.46	4.92E-09
9	453175	454374	+	3-oxoacyl-[ACP] synthase (EC 2.3.1.41) FabV like	-2.74	1.13E-04

9	454877	455602	+	3-oxoacyl-[ACP] reductase (EC 1.1.1.100)	-2.39	4.66E-03
9	455602	456828	+	FIG138576: 3-oxoacyl-[ACP] synthase (EC 2.3.1.41)	-2.89	9.46E-05
9	456825	458126	+	3-oxoacyl-[acyl-carrier-protein] synthase2C KASI (EC 2.3.1.41)	-4.04	5.05E-08
9	458123	458581	+	FIG00456945: hypothetical protein	-3.22	6.05E-03
9	458599	459039	+	Excinuclease ATPase subunit	-3.94	1.31E-06
9	461934	463409	-	Glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49)	-2.52	3.39E-07
9	470646	472292	+	Oligopeptide transport system permease protein OppB (TC 3.A.1.5.1)	-2.35	1.74E-07
9	481716	482495	-	Putative FMN hydrolase (EC 3.1.3.-)%3B 5-Amino-6-(5'-phosphoribitylamino)uracil phosphatase	3.67	5.19E-08
9	482660	483568	+	Transcriptional regulator2C LysR family	5.22	1.65E-05
9	483717	484187	+	Universal stress protein family	15.22	0.00E+00
9	484485	485795	-	Isocitrate lyase (EC 4.1.3.1)	9.51	0.00E+00
9	485775	485975	+	hypothetical protein	2.24	4.37E-05
9	486214	487827	-	ATP-dependent RNA helicase NGO0650	3.94	0.00E+00
9	487804	487995	+	hypothetical protein	2.46	3.20E-08
9	488251	488520	-	Acyl-CoA-binding protein	2.05	5.25E-03
9	500851	501330	+	Glutathione peroxidase family protein	-2.68	1.77E-06
9	502983	503879	-	Protein-N(5)-glutamine methyltransferase PrmB2C methylates LSU ribosomal protein L3p	-2.88	1.83E-05
9	503918	505057	-	N-succinyl-L2CL-diaminopimelate desuccinylase (EC 3.5.1.18)	-2.70	4.21E-144
9	505142	505504	-	FIG138056: a glutathione-dependent thiol reductase	-4.42	3.99E-15
9	505506	506333	-	22C32C42C5-tetrahydropyridine-22C6-dicarboxylate N-succinyltransferase (EC 2.3.1.117)	-2.72	6.31E-34
9	509273	512791	+	Chromosome partition protein smc	-2.19	1.79E-08
9	512922	514151	+	Cell division protein	-2.70	9.17E-25
9	514486	516537	+	DNA ligase (EC 6.5.1.2)	-2.35	5.03E-18
9	517128	518069	-	tRNA pseudouridine synthase A (EC 4.2.1.70)	-2.07	1.27E-04
9	526470	527252	+	Undecaprenyl diphosphate synthase (EC 2.5.1.31)	-2.22	3.57E-24
9	533205	533675	+	Outer membrane protein H precursor	-2.79	3.16E-38
9	533699	534775	+	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase (EC 2.3.1.191)	-2.43	8.75E-15
9	535449	536237	+	Acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase (EC 2.3.1.129)	-3.02	3.27E-33
9	536241	537410	+	Lipid-A-disaccharide synthase (EC 2.4.1.182)	-3.01	6.43E-26
9	537407	538216	+	Ribonuclease HII (EC 3.1.26.4)	-2.56	3.46E-18
9	540478	542814	+	Phosphoenolpyruvate synthase (EC 2.7.9.2)	2.36	0.00E+00
9	542952	543395	+	Putative activity regulator of membrane protease YbbK	2.16	5.04E-07
9	546956	547078	-	hypothetical protein	2.87	1.62E-13
9	557607	561623	+	Malonyl CoA-acyl carrier protein transacylase (EC 2.3.1.39)	2.04	2.46E-06
9	563382	564062	-	hypothetical protein	8.44	2.11E-10
9	564024	564626	+	Cytochrome c oxidase polypeptide II (EC 1.9.3.1)	3.10	5.00E-05
9	564643	565788	+	Capsular polysaccharide biosynthesis/export periplasmic protein WcbC	4.30	2.37E-13
9	565869	566993	+	Capsular polysaccharide export system inner membrane protein KpsE	4.57	1.55E-12
9	566999	567787	+	Capsular polysaccharide ABC transporter2C permease protein KpsM	3.06	6.50E-04
9	567784	568488	+	Capsular polysaccharide ABC transporter2C ATP-binding protein KpsT	3.17	1.11E-05
9	585171	588500	+	Type IV fimbrial biogenesis protein PilY1	3.71	5.63E-04
9	600289	600453	+	hypothetical protein	4.87	6.16E-03

9	624295	624858	+	FIG00444824: hypothetical protein	16.76	7.50E-03
9	626387	627409	+	hypothetical protein	14.61	7.16E-15
9	644062	644838	-	Hydroxypyruvate isomerase (EC 5.3.1.22)	6.23	1.33E-03
9	645031	645669	-	Ribulose-5-phosphate 4-epimerase and related epimerases and aldolases	8.44	0.00E+00
9	645669	647048	-	FIG00641944: hypothetical protein	8.16	9.44E-15
9	647007	647135	+	hypothetical protein	7.27	3.24E-09
9	647113	648003	-	2-hydroxy-3-oxopropionate reductase (EC 1.1.1.60)	17.27	4.58E-10
9	648083	648790	-	Transcriptional regulator PhnF	12.92	0.00E+00
9	649986	650837	+	Transketolase2C N-terminal section (EC 2.2.1.1)	30.78	1.63E-04
9	715842	717026	+	Acetylornithine aminotransferase (EC 2.6.1.11)	-2.53	3.48E-10
9	663526	664128	-	Cob(I)alamin adenosyltransferase (EC 2.5.1.17)	-3.03	1.07E-10
9	664125	664721	-	Cobalamin biosynthesis protein CbiG	-4.33	8.90E-50
9	664954	665118	-	hypothetical protein	-2.73	6.86E-03
9	665398	666525	+	CobW GTPase involved in cobalt insertion for B12 biosynthesis	-17.72	5.86E-157
9	666532	670455	+	CobN component of cobalt chelatase involved in B12 biosynthesis	-11.65	3.00E-21
9	670452	671600	+	ChlI component of cobalt chelatase involved in B12 biosynthesis	-16.92	2.92E-16
9	671663	672289	+	ChlD component of cobalt chelatase involved in B12 biosynthesis	-3.71	7.51E-14
9	672356	674254	-	Cobalamin biosynthesis protein CbiG / Cobalt-precorrin-3b C17-methyltransferase	-2.75	1.55E-17
9	680733	681473	+	Cobalt-precorrin-4 C11-methyltransferase (EC 2.1.1.133)	-3.10	3.38E-13
9	685182	687422	+	Ferrichrome-iron receptor	-6.45	2.73E-04
9	691904	692587	+	L-lysine permease	2.50	2.81E-07
9	693359	693484	-	hypothetical protein	2.17	2.29E-05
9	693527	695035	+	Kumamolysin	2.27	0.00E+00
9	700446	700988	+	FIG00457444: hypothetical protein	2.28	2.51E-14
9	701188	702045	-	NAD synthetase (EC 6.3.1.5)	2.82	0.00E+00
9	702313	703353	+	FIG00460350: hypothetical protein	6.99	8.28E-06
9	703454	704335	-	Permease of the drug/metabolite transporter (DMT) superfamily	3.42	3.17E-10
9	705784	706683	-	ABC-type polar amino acid transport system2C ATPase component	-2.15	3.03E-04
9	714471	715586	-	2-aminoethylphosphonate:pyruvate aminotransferase (EC 2.6.1.37)	2.43	3.99E-03
9	715984	717072	+	ABC transporter2C periplasmic ligand binding protein	3.46	8.09E-03
9	723934	724500	+	HD phosphohydrolase-like protein	2.51	8.25E-03
9	415948	418200	+	DinG family ATP-dependent helicase YoaA	2.88	7.56E-10

7.2 Figures

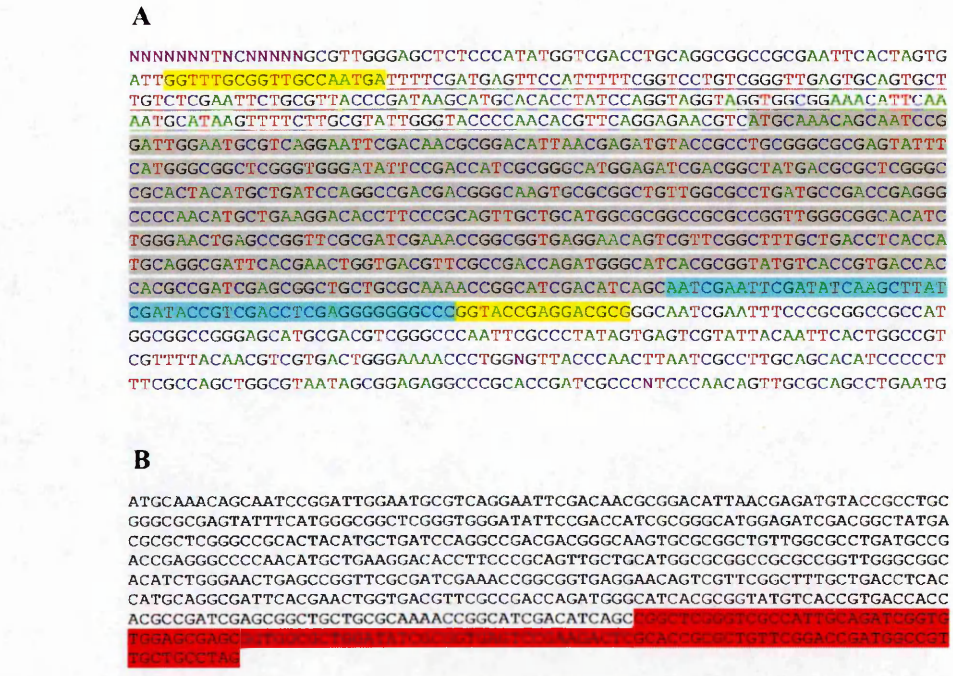


Figure 7.1. Confirmation of the construction of mutants PsJNBRAI, GR01BRAI and STM815BRAI. (A) Sequencing of PsJNBRAI. Genomic DNA of the mutant was used in PCR reactions using primers Phytof.braI.F and pKnock.old.R shown in yellow. The amplicon was then cloned in the pGEM®-T Easy vector and send for sequencing. Grey nucleotides represent the *braI* gene and blue ones represent part of the pKnock-Km plasmid. (B) Complete sequence of the *braI* gene of *B. phytofirmans* PsJN. The sequence highlighted in red represent the part of the gene that was separated from the rest by the insertion of pKnock-Km according to results showed in (A). (C) Construction of GR01BRAI and STM815BRAI as verified by Southern analysis. Lane 1 is "1 Kb Plus DNA Ladder", lane 2 is genomic DNA of *B. phymatum* GR01 digested with *Nde*I and lanes 3 and 4 are genomic DNA of GR01BRAI and STM815BRAI, respectively, digested with *Nde*I. These were then probed with the *braI* gene fragment obtained from the digestion of pBBRbraI$_{phym}$. In the parent strain (lane 2) a fragment of 2.8 kb was observed whereas in the mutants the size of the fragment is increased in 2 kb due to pKnock-Km insertion.

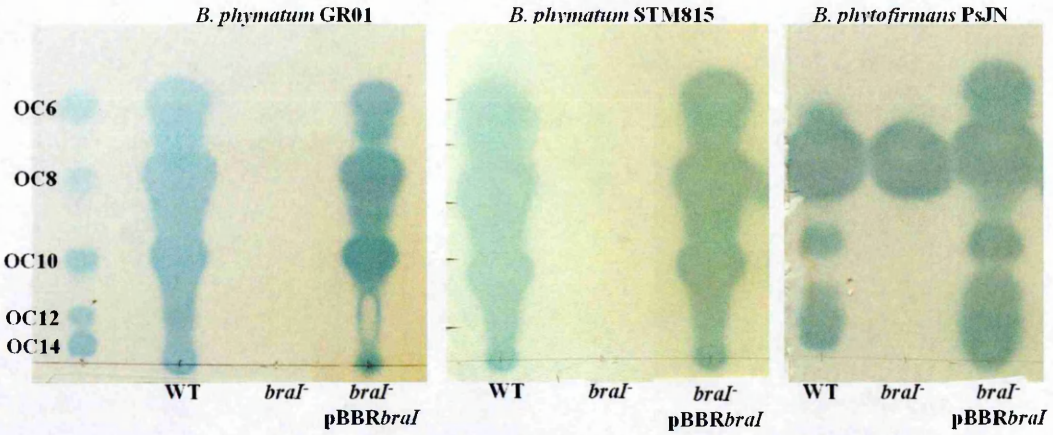


Figure 7.2. TLC analysis of the AHLs produced by the *Burkholderia* wild-type strains (WT), their QS mutant derivatives (*braI*) and the mutants complemented with the gene in pBBR plasmid (*braI* pBBR*braI*). AHL extraction was performed as described in Materials and Methods, and TLCs were performed in 70% methanol for 6 h. *A. tumefaciens* (pNTL4) was used to detect the AHL signals. Synthetic AHL compounds were used as a reference. OC6, 3-oxo-C_6-HSL; OC8, 3-oxo-C_8-HSL; OC10, 3-oxo-C_{10}-HSL; OC12, 3-oxo-C_{12}-HSL; OC14, 3-oxo-C_{14}-HSL.

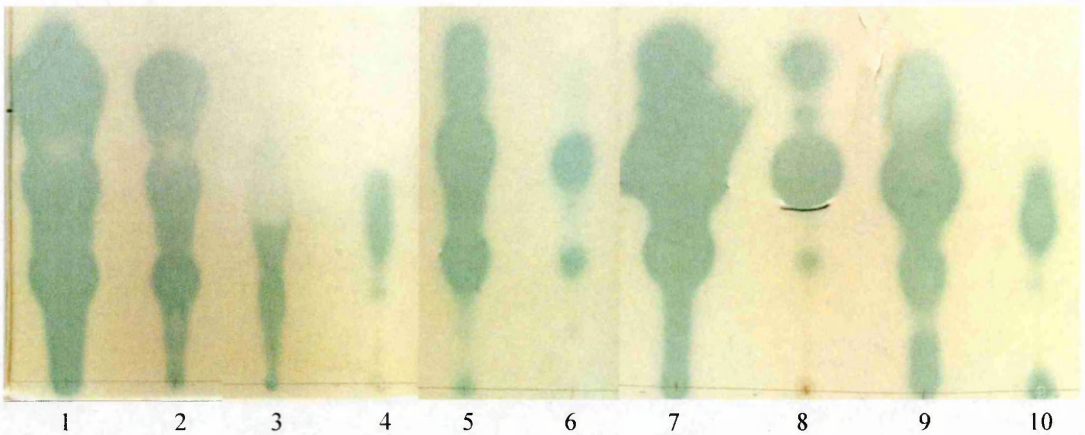


Figure 7.3. TLC analysis of the AHLs produced by the *Burkholderia* wild-type strains and mutant derivatives harboring the plasmid pME6863, which encodes for a lactonase through the *aiiA* gene. 1, *B.tuberum* DSM17489; 2, *B.tuberum* pME6863; 3, *B. graminis* DSM17151; 4, *B. graminis* pME6863; 5, *B. terrae* DSM17804; 6, *B. terrae* pME6863; 7, *B. phenazinium* DSM10684. 8, *B. phenazinium* pME6863; 9, *B. tropica* Ppe8; 10, *B. tropica* pME6863. AHL extraction was performed as described in Materials and Methods, and TLC was performed in 70% methanol for 6 h. *A. tumefaciens* (pNTL4) was used to detect the AHL signals.

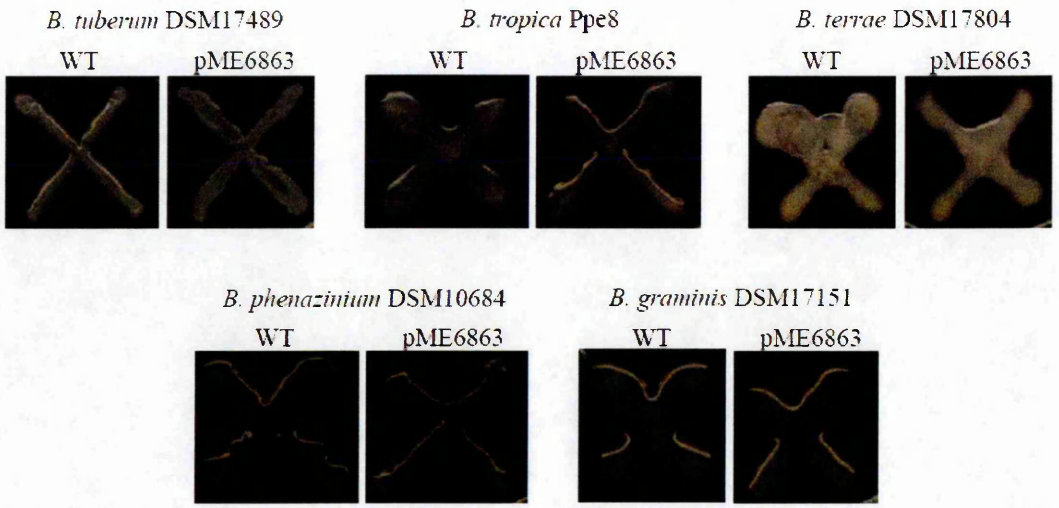
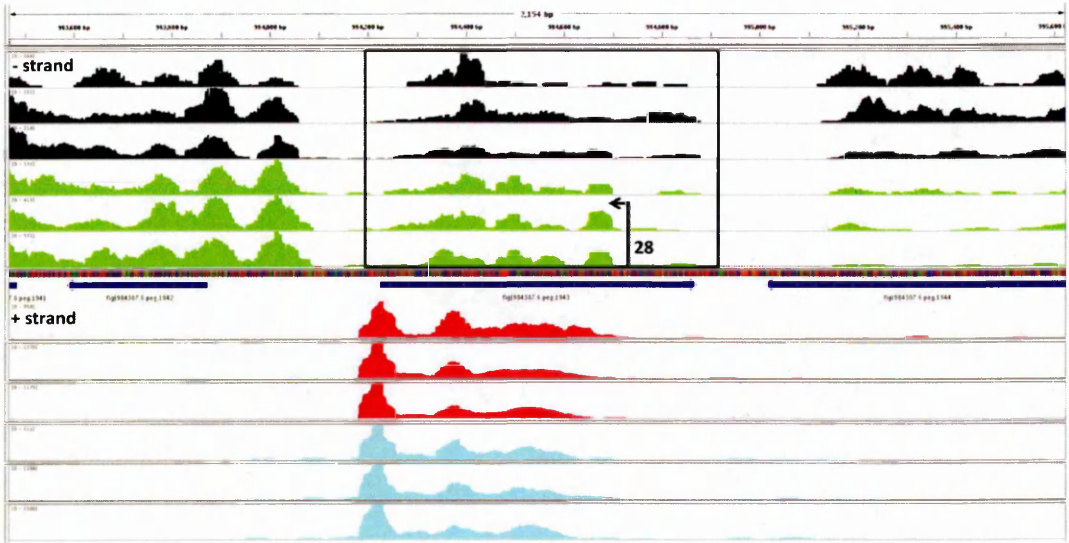


Figure 7.4. EPS production *of B. tuberum* DSM17489, *B. tropica* Ppe8, *B. terrae* DSM17804, *B. phenazinium* DSM10684, *B. graminis* DSM17151 and their respective QS defective strains harboring the plasmid pME6863, which possess the *aiiA* gene encoding for a lactonase. Single colonies were streaked in YEM, MM or NSA agar plates.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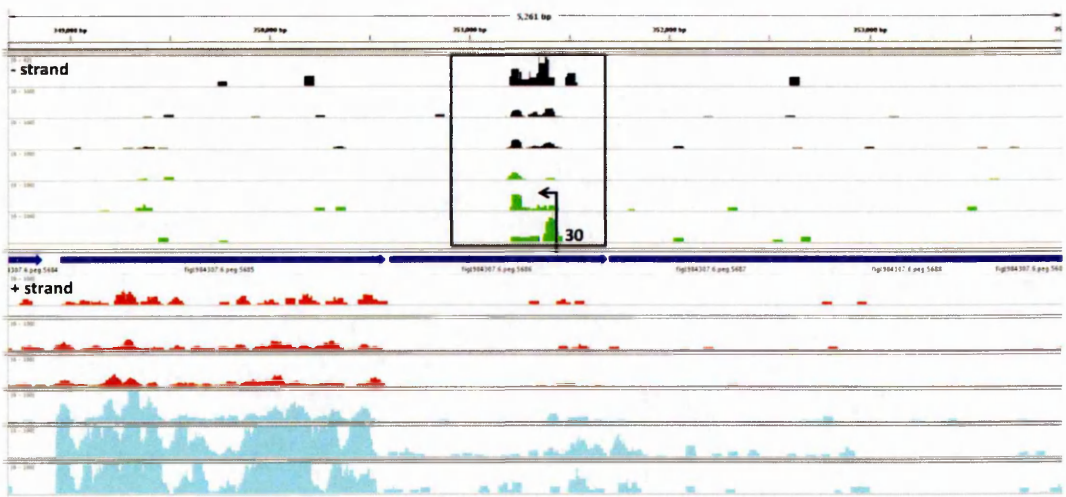
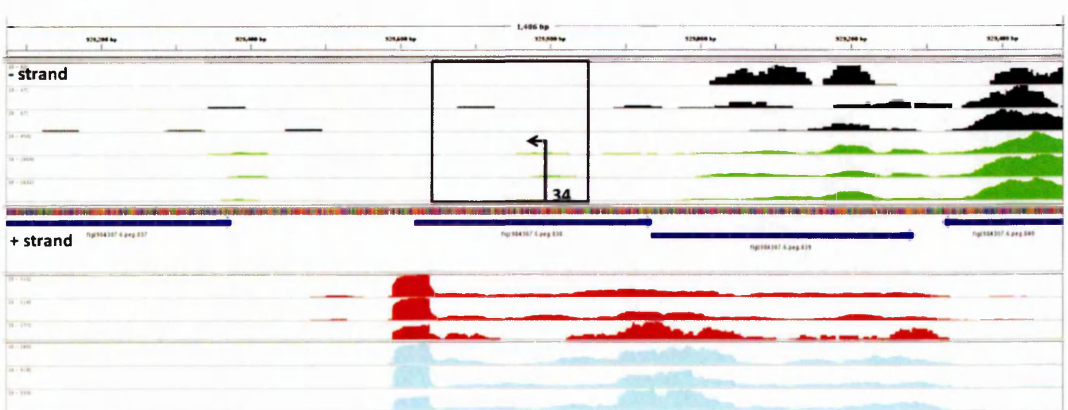
28
+ strand


- strand
30
+ strand


- strand
34
+ strand

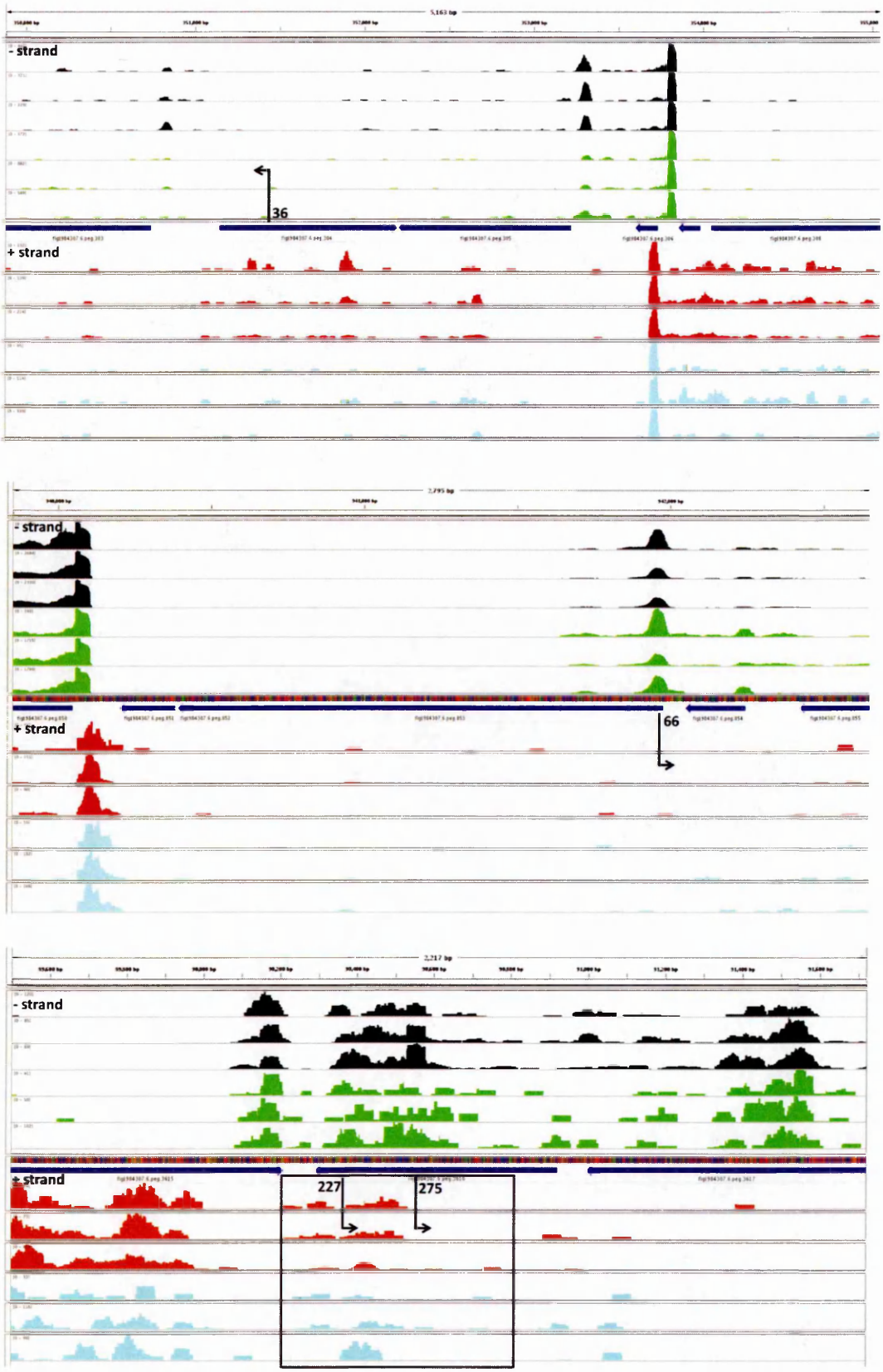

- strand
36
+ strand
- strand
66
+ strand
- strand
227
275
+ strand

Figure 7.5. Graphical representation of the cDNA reads identified by RNAseq in the regions of transposon insertions 28, 30, 34, 36, 66, 227 and 275. The transposon insertion site and the orientation of the *gusA* gene from mTn5-GusNm are identified with a black arrow; the black squares represent cDNA reads on the same orientation of the *gusA* gene; cDNA reads coloured in black or red derive from RNA samples isolated from *B. kururiensis* M130 grown in pure KB liquid medium and the green or blue ones derive from RNA samples isolated from the same strain grown in KB liquid medium added of rice plant macerate; the annotation of the genes was performed using the RAST server.